METMYOGLOBIN REDUCING ABILITY AND VISUAL CHARACTERISTICS OF NINE SELECTED BOVINE MUSCLES

A Dissertation

by

JASON MONROE BEHRENDS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2004

Major Subject: Animal Science

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Approved as to style and content by:

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ABSTRACT

Metmyoglobin Reducing Ability and Visual Characteristics of Nine Selected Bovine Muscles. (December 2004)

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Chair of Advisory Committee: Dr. Jeffrey W. Savell

Nine bovine muscles (*m. adductor*, *m. gluteobiceps*, *m. gluteus medius*, *m. longissimus lumborum*, *m. longissimus thoracis*, *m. psoas major*, *m. semimembranosus*, *m. supraspinatus* and *m. semitendinosus*) were removed from beef carcasses (n = 18) to study the affects of muscle, grade, and display time on color stability. Carcasses represented two USDA quality grades (Choice and Select) equally. Muscles were cut into steaks at 10 d postmortem and displayed under retail conditions for 7 d. Percent fat had a muscle × grade interaction as muscle reacted differently based on grade. The *m. gluteobiceps*, *m. gluteus medius*, and *m. semitendinosus* from Choice carcasses tended to be higher in percent fat than those from Select carcasses. Percent metmyoglobin increased from d 1 to d 7. High color stable muscles (e.g., *m. longissimus lumborum* and *m. longissimus thoracis*) displayed the least percent metmyoglobin. Generally most muscles displayed high metmyoglobin reductase activity throughout retail display. Aerobic reducing ability for low color stability muscles were high on d 1 and decreased dramatically by d 7, whereas high color stability muscles (*m. longissimus lumborum* and *m. longissimus thoracis*) showed an increase in aerobic reducing ability before

decreasing on d 7. Oxygen penetration depth was highest over the retail display in the two most color stable muscles (*m. longissimus lumborum* and *m. longissimus thoracis*). Myoglobin content decreased over time for all muscles despite differences in initial myoglobin concentration between muscles. TBARS values generally were a good indicator of discoloration and lean color. Color panel found that low color stability muscles decreased rapidly in lean color and increased in discoloration. Those muscles with poor color stability tended to have lower Hunter L* (lightness) and a* (redness). Muscle clearly had a major impact on overall color stability; however, grade showed only few differences, which conflicted with results from previous research. The USDA Select steaks tended to be higher in color stability than USDA Choice. Data indicated that differences in muscle types and grades play a major role in shelf-stability due to different oxidative potentials and reducing ability, and remain complicated factors in the quest to better understand color stability.

DEDICATION

To my parents

ACKNOWLEDGEMENTS

I extend my deepest gratitude to my family, especially my parents, Monroe and Karen (Dietert) Behrends, for their complete support, guidance, faith and love. I also thank my sister, Michelle (Behrends) Lowell, as well as my grandparents for their continued support and love.

I also express my thanks to my major professor, Dr. Savell, for all of his guidance and direction over the past three years. I have had the opportunity to learn and grow from the experiences I have been through. I also thank Dr. Miller for her support and guidance, as well as your knowledge and understanding. I also express my thanks to Dr. Keeton, for helping me prepare for the obstacles that presented themselves. I also thank Dr. Tarpley for his knowledge of anatomy and his continued quest for knowledge. I thank all my committee for teaching and giving me so many of the tools that will help on my journey through life.

I also thank all the undergraduate and graduate students that helped make this project a success. Without all of your support this project would not have been possible. Thank you all.

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CHAPTER I

INTRODUCTION

It is well known that muscle is a highly specialized tissue, and its function represents a classic example of conversion of chemical to mechanical energy in a living system. Understanding the physiological and structural components of muscle is essential to comprehending the effects they have on overall color stability. It is necessary to understand the biochemical factors that influence color stability and the formation of metmyoglobin in beef muscles.

Renerre and Labas (1987) found meat color stability and oxidation-reduction potential of myoglobin are highly related to muscle type. Renerre and Labas (1987) also found that enzymatic ferrimyoglobin reduction under aerobic conditions (metmyoglobin reductase activity) does not fully explain the differences observed in muscle color stability. It is quite clear that muscle tissue is a very complex material that depends on many factors. O'Keeffe and Hood (1982) reported that color stability may be a function of oxygen consumption rate by muscles postmortem, depth of oxygen penetration into the tissues, rates of myoglobin oxygenation and deoxygenation and myoglobin content, and succinate dehydrogenase activity. These factors play a major role in color stability and are extremely different between muscles.

Lipid oxidation and metmyoglobin formation may occur simultaneously and lipid oxidation can accelerate the conversion of myoglobin to metmyoglobin. Numerous

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This dissertation follows the style of Journal of Animal Science.

factors affect lipid oxidation including intensity and spectra light, oxygen concentration, temperature, presence of anti- and pro-oxidants, degree of fatty acid unsaturation, and presence of enzymes (Skibsted et al., 1998). Lipid oxidation is not normally considered to limit shelf-life of aerobically packaged, refrigerated meat, since lipid oxidation occurs at a slower rate than discoloration or microbial growth (Zhao et al., 1994).

There have been numerous attempts to extend the shelf-life of fresh meat products. One of the most promising treatments involves dietary supplementation with Vitamin E, which has been shown to be an effective means of extending the stability of color and lipids in fresh meats (Arnold et al., 1993). The function of Vitamin E is not fully understood; however, it does increase alpha-tocopherol levels, which does act as an oxygen scavenger, helping to prevent metmyoglobin formation and lipid oxidation. Studies have focused on few muscles (i.e. *m. longissimus dorsi*, *m. gluteus medius*, and *m. psoas major*) with regard to their susceptibility to oxidation. As the industry continues to identify individual muscles through muscle profiling, it is essential to understand the properties of these muscles when displayed in the retail case.

The purpose of this study was to study the affects of muscle, grade, and display time on color stability. Understanding individual muscles more will allow the meat industry to utilize muscles differently. The objective is to identify basic components of different muscles to clearly enhance and understand color stability.

CHAPTER II

REVIEW OF LITERATURE

Meat color is a complicated system and the largest contributor to meat color is myoglobin. This protein is a very dynamic structure that undergoes color changes rather easily. Myoglobin contains a protein portion and a non-protein portion. The non-protein portion contains a heme ring and at the center of the heme is a Fe^{2+} atom. Four of the six coordintation sites around this atom are occupied by nitrogen atoms from a planar porphyrin ring. The fifth coordination site is occupied by a nitrogen atom from a histidine side chain on one of the amino acids in the protein. The color state of meat is determined, for the most part, by last coordination site and the redox state of the iron atom (Fe^{+2} or Fe^{3+}). For fresh meat when the sixth site binds to H_2O and is in the Fe^{+2} state, the color of the meat is purple and commonly referred to as myoglobin (deoxymyoglobin). When the sixth site binds to O_2 and is in the Fe^{+2} state, the color is referred to as oxymoglobin, and when the sixth site binds to H_2O and is in the Fe^{+3} state the color of the meat is brown and is commonly referred to as metmyoglobin. Metmyoglobin is the most stable of the three mentioned and is the primary cause of deterioration and discount seen in the retail case, as well as consumer elimination of product. The last coordination site and the Fe state are the first links to color. Early post-mortem of myoglobin to oxymyoglobin and metmyoglobin is shown in Figure 1. Late post-mortem conversion of myoglobobin to oxymyoglobin and metmyoglobin is shown

in Figure 2. Colors of boxes refer to activity (Green = high activity; Blue = Neutral; Red = Low activity).

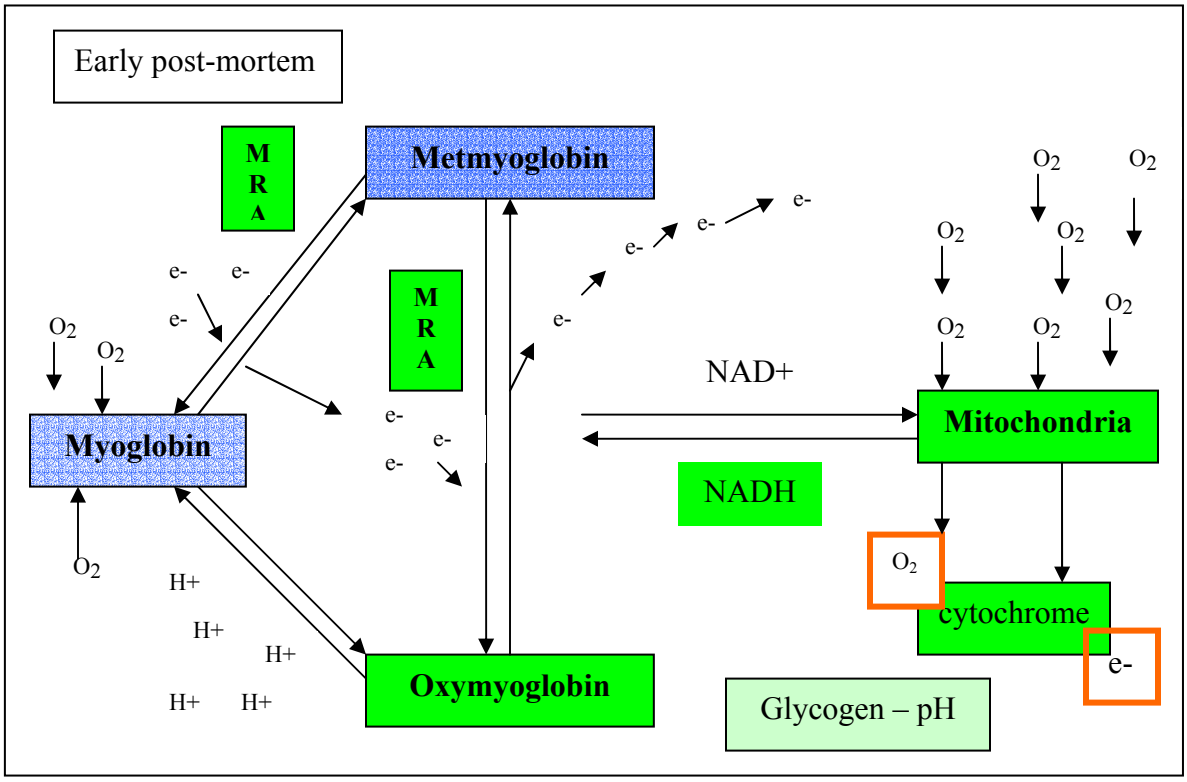

Figure 1. Early post-mortem metmyoglobin reducing ability (Green = high activity; Blue = Neutral; Red = Low activity)

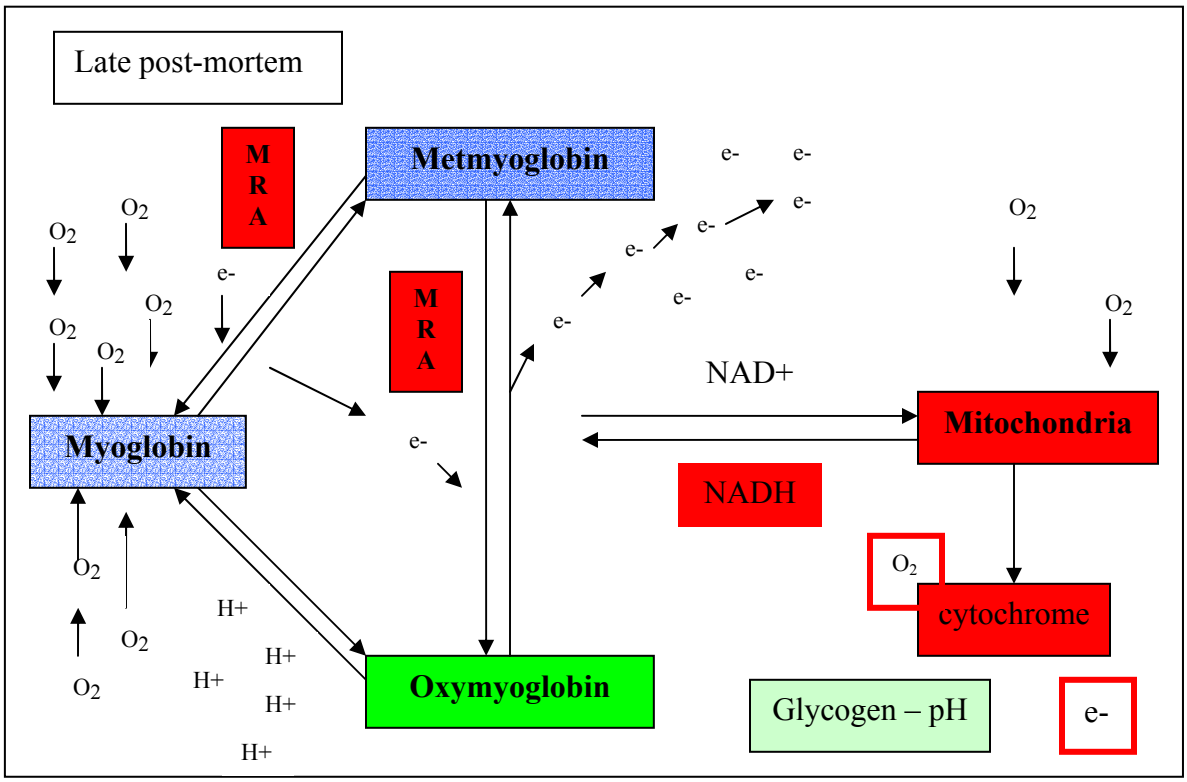

Figure 2. Late post-mortem metmyoglobin reducing ability (Green = high activity; Blue = Neutral; Red = Low activity)

Industry

As economic pressures on the meat industry continues to mount, the advantages of centralized cutting and packaging of all meat products prior to the retailer as case-ready products becomes more evident (Griffin et al., 1982b). Voltz and Marsden (1963) identified numerous advantages for centralized prepackaging of meat in a 40 store operation utilizing cuts wrapped in oxygen-permeable polyvinyl chloride (PVC) film. A limiting factor of their system was the short period of product acceptability (3-4 days) after cutting and packaging.

Research has been limited on the use of vacuum packaged retail cuts in a centralized distribution system because the appearance, especially the lean color, of meat in the retail case which is a major factor in consumer acceptance (Kropf, 1980; Walker, 1980). Ninety percent of all beef in the United States presently leaving packing plants is in the form of vacuum-packaged subprimal cuts. Many reports (Griffin et al., 1982a; Boers et al., 1994; Bell and Garout 1994; Unsal et al., 1995; Sahoo and Anjaneyulu, 1997) have indicated that vacuum packaging of meat prolongs shelf-life as compared with that of cuts packaged in oxygen permeable film. The purple-red color of the reduced myoglobin pigment found in vacuum packaged cuts (Ernst, 1980; Kropf, 1980; Lawrie, 1979) is limiting factor in consumer acceptance of vacuum packaged retail cuts. Short time intervals between cutting and packaging, along with extremely low partial pressures of oxygen in the package, also must be achieved to ensure against formation of metmyoglobin. Ernst (1980) reported that although consumers commented on the dark lean color of vacuum packaged retail cuts as compared with the cherry-red color of PVC wrapped cuts, when this color phenomenon was understood by consumers, the dark lean color was not reported as a deterrent to sales.

Metmyoglobin is associated with the brown lean color and most often equated with spoilage in fresh meat by consumers (Ernst, 1980). Jeremiah and Carpenter (1972) indicated that consumers do not prefer steaks that are extremely dark or extremely pale in muscle color. Griffin et al. (1982a) found that individual muscles from beef round steaks have unique lean color characteristics when vacuum packaged.

The industry continues to search for ways of increasing color shelf-life of beef without giving up the bright cherry red appearance of meat in its oxymyoglobin state. There are clearly differences between species (Millar et al., 1994), in addition to differences between muscles (Behrends et al., 2003). What makes these muscles different is not fully understood. By focusing on the biochemical aspect of muscle, we can target specific muscles differently based on the increased understanding of muscle color. Consumers continue to demand that fresh beef be a bright cherry red color for an extended period of time. With the increased use of case-ready meats, there is an increase need to understand basic meat components that contribute to color stability. The introduction of case-ready modified atmosphere packaging has increased shelf-life of fresh meat products; however, some muscles react differently to MAP (Behrends et al., 2003). Behrends et al. (2003) found that round muscles react differently when placed in a high oxygen modified atmosphere. They found that the *m. semitendinosus* was more color stable than the *m. biceps femoris* and *m. semimembranosus.* Obstacles still exist regarding the color of the product in the reduced myoglobin and increased metmyoglobin states due to beef's higher myoglobin content versus other species, such as pork, which is less drastically affected.

Over the past few years, Modified Atmosphere Packaging (MAP) has become an important component of the meat industry as a method to provide a more color shelf-stable product. It is quite clear that there are numerous factors that affect color stability of meat. There have been studies that report that pork is the most stable, followed by

beef and then lamb. These differences help to provide information that it is a variety of components of muscle that effect color stability of different muscles.

For years muscle profiling of muscle has been done to evaluate the usefulness of the specific muscle (Belew et al., 2003). Muscle profiling is a marketing tool in an attempt to increase the value of the product. By selecting specific muscles that are commonly used for steaks and roasts, we will be able to evaluate the color stability of these muscles and evaluate the specific components of muscle that contribute to color stability.

Color Shelf-life

Consumers have become accustomed to a desirable color of fresh beef that is bright red and that any deviation from this is deemed unacceptable. This cherry red color of fresh beef is due to oxymyoglobin content when exposed to oxygen. Millar et al. (1994) reported oxymyoglobin formation (blooming) occurs more rapidly in beef than pork or chicken. There was little evidence of oxymyoglobin formation in chicken muscles although formation was evident in the pork samples and after 24 h storage the muscles oxidized to produce some metmyoglobin at their surface.

Vrana et al. (1985) reported differences in pork loin chops packaged in different oxygen barrier films. Lean color scores from chops from various storage treatments displayed in two different retail packaging systems. Vrana and associates (1985) also stated that loin storage treatment made little, if any practical difference in lean color scores for PVC wrapped chops displayed for up to 4 d or for vacuum-packaged loin chops displayed for up to 10 d. Their data suggested that within the retail packaging

system, the method of loin storage did not appear to greatly affect lean color. However, they found significant differences in surface discoloration, with higher discoloration scores in PVC-wrapped and parchment paper-wrapped storage treatment products than those in VP storage treatments.

Metmyoglobin

The function of the production of metmyoglobin has been studied for years. The accumulation of metmyoglobin on the surface of fresh beef is a major deterrent to consumers. The phenomenon of metmyoglobin on meat is dependent on several mechanisms, which includes the rates of oxygen diffusion and oxygen consumption (Bendall and Taylor, 1972; Atkinson and Follett, 1973), the autoxidation of the pigment in the presence of oxygen (George and Stratman, 1952; Brown and Mebine, 1969), and the enzymatic reduction of metmyoglobin (Stewart et al., 1965; Watts et al., 1966).

Metmyoglobin has been examined by many (Luno et al., 1998; Solberg, 1970; Greene et al., 1971), and it has been measured as one of the indicators of color stability. Understanding what makes one muscle convert more myoglobin to metmyoglobin is a very important aspect to evaluate. Greene et al. (1971) reported that meat with as little as 40% metmyoglobin caused meat to be rejected by consumers. Renerre et al. (1996) reported that metmyoglobin and lipofuscin contents were higher in color-unstable muscles, such as the *m. psoas major* and *m. diaphragma* versus the *m. longissimus lumborum* and *m. tensor fascia latae*. Lipofuscin is classified as the "aging" pigment. This is the pigment left over from the breakdown and digestion of damaged blood cells. Renerre et al. (1996) found that the *m. psoas major* and *m. diaphragma* had higher

antioxidant enzymes; however, that was insufficient to prevent increased formation of metmyoglobin and lipofucin in these muscles compared to the *m. longissismus lumborum* and *m. tensor fascia latae*.

Along with the biochemical factors that affect muscle color, other retail properties can greatly affect the shelf-life of beef, including temperature (Jeremiah and Gibson, 2001). They found that when temperature increased, metmyoglobin formation increased along with surface discoloration. Gill and McGinnis (1995) evaluated effects of residual oxygen concentration and temperature on degradation of the color of beef packaged under oxygen depleted atmospheres. All longissimus dorsi samples stored for 12 h at 5 to 1°C were extensively discolored, with metmyoglobin fraction generally exceeding 60%, but those stored at -1.5°C for 48 h or less, under O_2 concentration < 400 ppm, displayed less discoloration. However, even with the subzero temperature (-1.5°C), after 12 h or longer storage, the samples from N_2 atmospheres were generally assessed as being of undesirable color, with extensive discoloration and metmyoglobin fraction in excess of 50%. Moreover, Gill and McGinnis (1995) reported all samples stored under N_2 at 0°C for 48 h were of undesirable appearance and extensively discolored, with greatly increased metmyoglobin fractions from those stored for 24 h.

There are numerous factors that affect the rate of metmyoglobin accumulation in pre-packaged beef. Hood (1980) reported that inter-muscular variability is one of the most important factors when it comes to color stability. In addition, Hood (1980) reported that age postmortem and inter-animal variability also can affect color stability.

Hood (1980) reported that the *m. longissimus* was the most stable, followed by the *m. semimembranosus* and *m. gluteus medius* with the *m. psoas major* as the least stable.

Color Wheel

It is important to discuss the major factors thought to contribute to color stability. These factors may react differently between muscles; however, research has yet to fully explore the synergistic affect of multiple factors on color stability. The color wheel will be discussed to spread light on research that has been done to help clarify the mechanism of color and how each muscle has differing color stability.

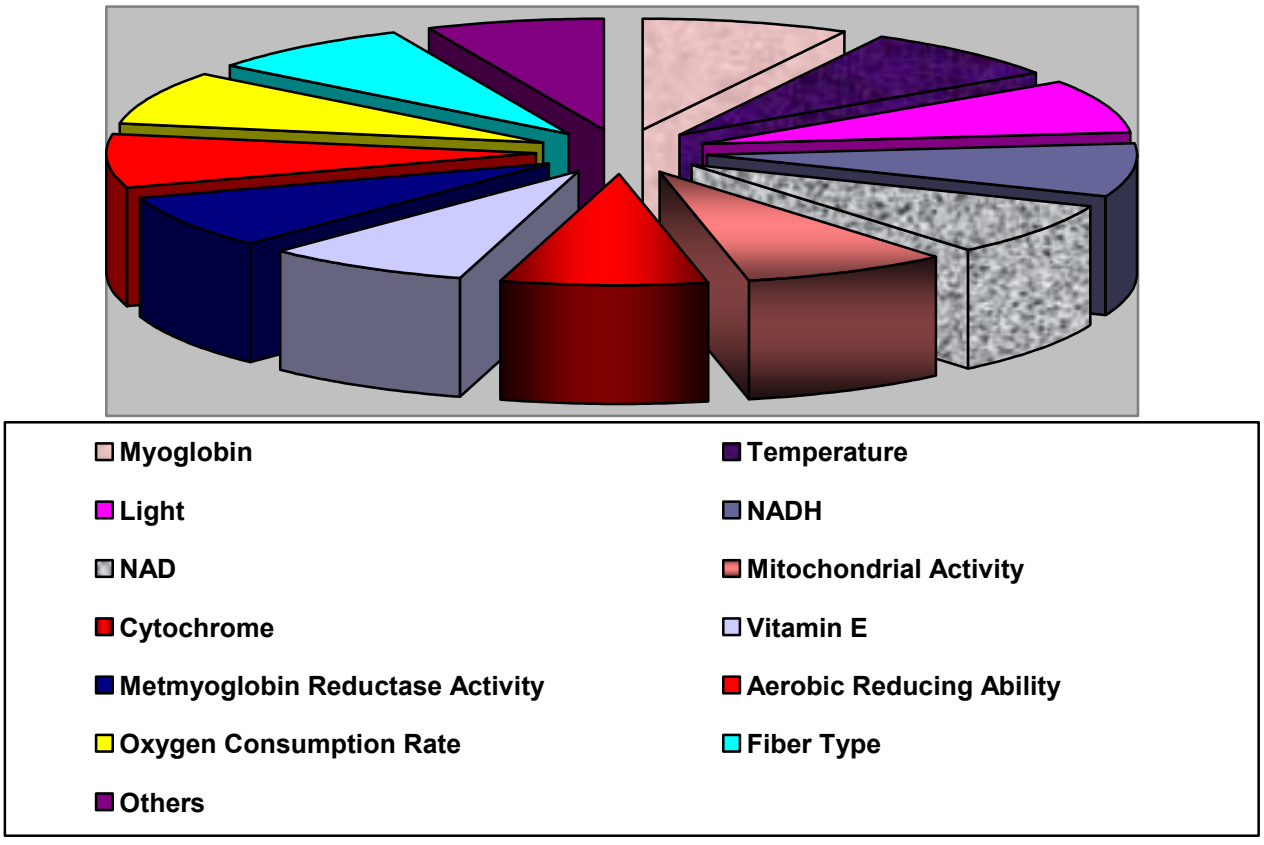

Figure 3. Color wheel

Researchers continue to find that no one mechanism in muscle controls color stability. Through the years, myoglobin content, temperature, light, NADH, NAD, mitochondrial activity, cytochrome, vitamin E (alpha-tocopherol), metmyoglobin reductase activity, fiber type, as well as many others have been studied to better assess color stability.

Myoglobin

Conversion of myoglobin to oxymyoglobin is an important factor to evaluate. The time it takes a muscle to convert myoglobin to oxymyoglobin may be a direct factor in the amount of time it takes to convert to metmyoglobin. O'Keeffe and Hood (1982) reported that *m. psoas major* was two to three more times efficient in converting oxymyoglobin back to myoglobin than *m. longissimus*. This allows for faster conversion to the intermediate metmyoglobin. O'Keeffe and Hood (1982) also found that myoglobin content was higher in *m. longissimus* than the *m. psoas major*.

Heme and Non-heme Iron. Heme iron content in meat is of utmost importance because it allows us to determine the storage stability of these food products since iron released from heme pigment by heating is one of the major catalysts of lipid oxidation (Love and Person 1974; Igene et al., 1979). Lombardi-Boccia et al. (2002) evaluated total heme and non-heme iron contents in numerous species. They found that heme iron in red meats ranged from 72 to 87%, while in rabbit and pork it was 56 and 62%, respectively. The importance of heme and non-heme iron is not only for nutritional aspects, but they play an important role in color stability.

Renerre and Labas (1987) reported no significant differences in haeminic iron content between the *m. psoas major* and the *m. tensor fasciae latae*. Stewart et al. (1965) reported significant correlations between haeminic iron concentration and reducing activity.

In certain aging processes, bacterial growth is inhibited by allowing evaporation from the meat surface to occur. Under these conditions meat takes on a dark, dull appearance due presumable to increased heme concentration and structural change (Brooks, 1937). It also has been observed (Ledward, 1970) that semitendinosus muscles of normal water content, from different animals, tend to develop different levels of metmyoglobin during storage, the level developed being independent of the muscle pH.

Temperature and Light

Temperature is a deterrent for color stability. As temperature increases, color stability decreases. Retail cases temperatures tend to fluctuate, which exposes meat to possible temperature abuse. This temperature abuse speeds up color conversion from oxymyoglobin to metmyoglobin and thereby, decreasing color stability. Hutchins et al. (1967) found that the rate of metmyoglobin reduction decreased with decreasing temperatures; however, they also reported an increase in metmyoglobin formation at higher temperatures, as myoglobin would be oxidized more rapidly. Hood (1980) found that metmyoglobin content increased as temperature increased for all muscles.

In addition, UV from light can produce discoloration in all muscles. Kropf (1980) stated that display lighting could cause discoloration due to temperature increases on the meat surface, photochemical effects, or differences in light rendition. Light tends

to decrease color stability faster than product stored in the dark. Kennick et al. (1971) reported beef steaks held under lighting deteriorated faster than those held in the dark. Kropf (1980) stated lighting may not affect color early in retail display, but may be a factor as storage progresses. Hood (1980) found that fluorescent light and UV increased discoloration in *m. psoas major*, *m. semimembranosus*, *m. vastus lateralis*, *m. gluteus medius*, and *m. semitendinosus* when compared to those that were stored in the dark.

NADH and NAD+

Jerez et al. (2003) reported that muscles treated with various solutions showed higher reduction-oxidation potential and lower NAD+ content (higher oxidizing capacity) values than the control at 24 h post-mortem. They also presented data that showed the decline in reducing capacity during storage appeared to occur more slowly in treated muscles than controls. Jerez et al. (2003) characterized the reducing conditions (redox potential, NAD+ and NADH content) among treatments because they are likely to affect color. They found that for most muscles glycolytic inhibitors (NaA, NaC, and NaF) resulted in the highest NAD+ concentrations ($P< 0.05$), but these differences were not of sufficient magnitude to be reflected in redox potential and no differences were found in NADH content in the study by Jerez et al. (2003).

Faustman and Cassens (1991) found that NAD concentration decreased during storage, along with increased deterioration of color. They stated that NAD decline may be due to enzymatic action, which could contribute to NAD hydrolysis. Atkinson and Follett (1973) reported that decreasing endogenous NAD could increase color stability; however, Faustman and Cassens (1991) found that the less color stable muscle (*m.*

gluteus medius) had less NAD than the more color stable, *m. longissimus*. Clearly, there is a need to assess overall oxidative potential to understand the role NAD plays in the game of color stability.

The use of NADH to measure metmyoglobin reductase activity is essential. It is known that NADH is a rate limiting step in metmyoglobin reductase activity. Reddy and Carpenter (1991) found that when NADH was left out of the assay, there was no metmyoglobin reductase activity found. Much discussion has been made at the usefulness of the current procedure utilizing NADH because muscles do not carry the same amount of NADH. Thus, adding NADH at equal amounts may alter the outcome of metmyoglobin reductase activity.

Mitochondrial Activity

Mitochondrial activity obviously plays a major role in muscle. As a muscle ages post-mortem, mitochondrial activity decreases. This may play a more important role than previously thought. When mitochondrial activity decreases, there is a lack of conversion of energy, which decreases the metmyoglobin reducing ability. Mitochondrial activity in post-mortem muscles is increased by high storage temperature and high pH values (Ashmore et al., 1972; Bendall, 1972; Bendall and Taylor, 1972; Cheah and Cheah, 1971; Cornforth and Egbert, 1985). Oxygen in meat is used for oxygenation of the meat pigment or/and mitochondrial respiration (Lanari and Cassens, 1991). Therefore, as mitochondrial activity decreases, oxygen is consumed at a higher rate by meat pigments.

Gasperlin et al. (2000) reported the dark color of DFD beef is directly dependent on high mitochondrial respiration. Mitochondrial oxidation is approximately 50 – 70%

higher at pH of 7.2 than at pH 5.8 (Bendall and Taylor, 1972). The high mitochondrial respiration maintains a lower concentration of oxymyoglobin, and Lawrie (1958) concluded that the increased oxygen consumption and decreased availability of oxygen in this meat increased the concentration of dark-red deoxygenated myoglobin.

Cytochrome

There have been evidence that cytochrome could play a role in color stability. It has been shown that cytochrome may act as an oxygen scavenger, inhibiting metmyoglobin formation. There have been studies that have shown there are different types of cytochrome and one may be more important in color stability.

Schenkman and Jansson (2003) reported that cytochrome b_5 may impose a positive modified action of cytochrome P450 monooxygenase reaction. They report that cytochrome b_5 is known to be involved as an electron transfer component in a number of oxidative reactions in biological tissue.

Watts et al. (1966) theorized the effects of cytochrome on conversion of metmyoglobin to myoglobin. Watts et al. (1996) hypothesized a scheme that is described below. They evaluated the affect of added DPN and succinate and of known inhibitors on reducing activities of meat. Four inhibitors were used in their study. Oxalate acted as a competitive inhibitor for lactate in the LD region (Ottolenghi and Denstedt, 1958), thus blocking the overall reaction in position 1, provided lactate is the substrate. Watts et al. (1966) reported amytal and rotenone both block reduction of DPNH in flavoprotein region, position 2, but have no effect on succinate oxidation. Antimycin A blocks electron transport from DPNH of succinate at position 3. Unfortunately, Watts et al.

(1966) stated inhibitors in the cytochrome A region are compounds such as cyanide, azide, etc., which combine with heme iron and therefore react with myoglobin and metmyoglobin, as well as with cytochrome A.

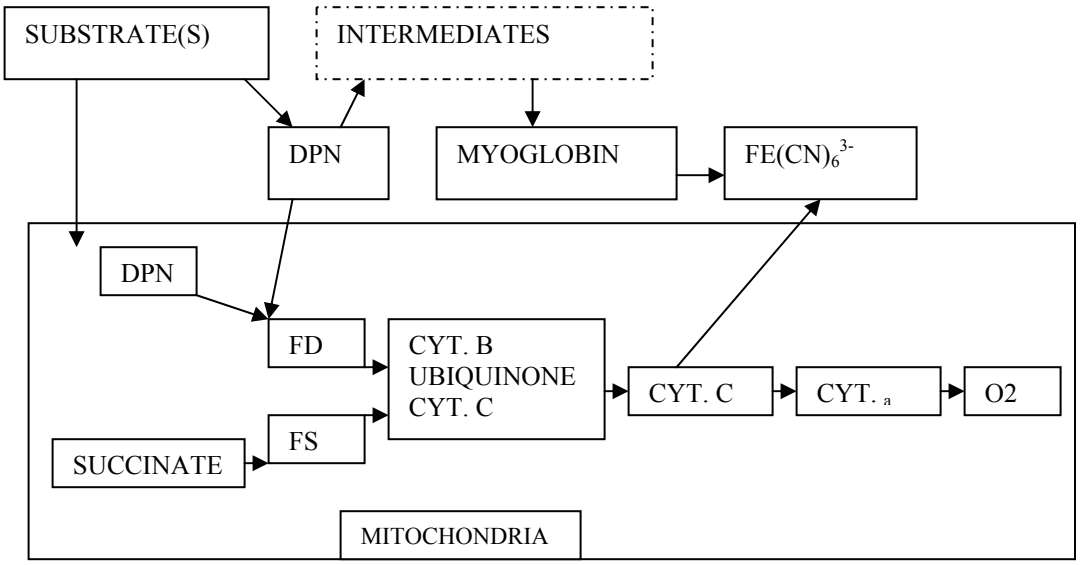

Figure 4. Electron transfer through cytochrome (Watts et al., 1966)

Vitamin E

The function of vitamin E is still not fully understood. It is believed that vitamin E increases the already naturally occurring antioxidant, alpha-tocophorol levels. This antioxidant helps to prevent oxidation of lipids (O'Grady et al., 1998), as well as myoglobin, thus preventing the onset of metmyoglobin (Chan et al., 1998). For years research has been conducted to evaluate the shelf-life of fresh meats. Supplements have been fed to live animals to hopefully increase shelf-life and produce a more stable color. Faustman et al. (1998) reported muscles vary in their color stability, and this relative

change is not changed by vitamin E supplementation, but instead through an interaction between alpha-tocopherol, lipid oxidation, and oxymyoglobin oxidation. They reported alpha-tocopherol seems to exert its color stabilizing effect by indirectly delaying oxymyoglobin oxidation via direct inhibition of lipid oxidation.

Sanders and associates (1997) examined vitamin E in cattle as a possible extender of shelf-life. There was less discoloration in vitamin E treated steaks versus control steaks. In addition, vitamin E also decreased lipid oxidation prolonging shelf-life. Extension of beef color shelf-life by dietary vitamin E supplementation could provide a significant economic return. It was estimated that increasing the color shelf-life of beef by 2 days would save $792 million annually in US retail beef sales (Liu et al., 1995).

Zanardi et al. (1998) evaluated the oxidative stability of fresh and cooked pork chops and found greater color stability in meat packaged under modified atmosphere with increased vitamin E content. However, the same did not apply to meat packaged in oxygen permeable film. No differences were observed in fatty acid oxidative stability of fresh meat longissimus dorsi and cooked chops (peroxide and TBARS values) (Zanardi et al., 1998).

Chan and associates (1996) evalutated the effects of dietary vitamin E on color stability and sensory assessment of spoilage in three beef muscles. They found color stability of three muscles followed the order of *m. longissimus lumborum* > *m. gluteus medius* > *m. psoas major*. Vitamin E-treated muscles had less metmyoglobin formation, higher a* values (redness) and lower hue angle values than controls during storage at 4°C. In addition, Chan et al. (1996) presented data indicating sensory panelists preferred

the appearance of vitamin E-treated beef steaks. Dietary vitamin E supplementation delayed oxymyoglobin oxidation of the three muscles and increased the color shelf-life without affecting total microbial load.

O'Grady et al. (2001) found that inclusion of alpha-tocopherol helped to reduce lipid oxidation and oxymyoglobin oxidation in *m. longissimus*. O'Grady et al. (1998) reported that oxymyoglobin oxidation was only significantly accelerated when a threshold level of lipid oxidation had been exceeded.

Studies have been done to evaluate the effects of forage versus grain fed cattle, on the levels of alpha-tocopherol content. O'Sullivan et al. (2003) reported no significant differences between diets; however, they found a trend that cattle that were forage fed tended to have higher levels of alpha-tocopherol in the *m. longissimus*.

Metmyoglobin Reductase Activity and Aerobic Reducing Ability

There have been many studies which have evaluated the importance of metmyoglobin reductase activity (MRA). This enzyme helps convert metmyoglobin back to myoglobin. The reducing ability of MRA is an important biochemical factor to examine when looking at color stability.

According to Renerre and Labas (1987), the loss of anaerobic MRA in post-rigor meat is due to factors such as decreases in tissue pH, depletion of substrates and cofactors such as coenzymes (NADH), oxidative deteriorative changes, and decreasing enzymatic activities including disintegration of mitochondrial particles.

Ledward (1972) found that aerobic reducing ability is an important measurement of metmyoglobin accumulation. Along with metmyoglobin reductase activity, Ledward

(1972) reported a method to evaluate aerobic reducing ability, as a measure of MRA. Ledward (1972) reported the MRA and aerobic reducing ability may be measuring different reduction systems.

Conflicting results have been reported for MRA, where Atkinson and Follet (1973) reported MRA was higher in lamb than beef or pork despite being less color stable. While O'Keeffe and Hood (1982) found high MRA was higher those muscles that were more color stable. Then again, Renerre and Labas (1987) observed the highest MRA in the least color stable muscle, *m. diaphragma*.

Oxygen Consumption Rate

O'Keeffe and Hood (1982) reported that differences of oxygen consumption rate and myoglobin content greatly effects the conversion of oxymyoglobin to metmyoglobin. They found that the *m. psoas major* has a high oxygen consumption rate in addition to a high conversion of oxymyoglobin to metmyoglobin. O'Keeffe and Hood (1982) reported that the most significant factor affecting color stability of beef muscles appears to be their enzymatic activity which determines the rate of myoglobin oxidation. Oxygen consumption is directly related to mitochondrial activity in muscle as previously discussed. Therefore, measuring oxygen consumption rate can help determine mitochondrial activity between muscles.

It has been reported that oxygen consumption in muscles decreases exponentially during the first 6 days of storage at 2°C and then remains approximately constant (Bendall and Taylor, 1972). Others have found different results when the exponential decrease starts or the time at which oxygen consumption remains unchanged (Atkinson et

al., 1969; Carafoli and Gazzotti, 1970; Cheah and Cheah, 1971). O'Keeffe and Hood (1982) and Renerre and Labas (1987) reported that muscles with greater discoloration and low color stability displayed higher rates of oxygen consumption.

Fiber Type

Muscle fiber type composition, fiber areas and capillary density of specific muscles are important factors influencing many of the peri- and post-mortal biochemical processes and thereby meat quality (Klont et al., 1998). Muscle fiber typing is a difficult and very tedious procedure. This makes identifying shelf-stability solely on the bases of fiber type extremely difficult. There have been reports that fiber type effects palatability (Melton et al., 1975), marbling (Calkins et al., 1981), muscle growth (Johnston et al., 1981) and quality and yield grades (Calkins et al., 1981; Melton et al., 1974). Muscle fiber type has been shown to be related to breed, sex and method of feeding (Johnston et al., 1981). They found that when using different feeding systems different amounts of fiber types occurred and when there was an increase in muscle bundle size there was an increase in beta fiber population diameter.

Kirchofer et al. (2002) evaluated fiber-type composition of muscles from the chuck and round. While it has been long believed that white muscle fibers have a lower oxidative potential, Kirchofer and associates (2002) found that majority of the muscles evaluated were classified as white fibered. However, it is the proportion of oxidative (beta-red) fibers that is important when assessing color stability. Kirchofer et al. (2002) stated oxidative (beta-red) fibers will have a high concentration of mitochondria, there by increasing its oxidative activity and reducing color stability.

Calkins et al. (1981) found that increases in white fiber types decreased marbling. They also found that there were higher marbling scores with increased amount of red oxidative muscles. Calkins and associates (1981) reported that oxidative capacity of a muscle is related to marbling and tenderness and that muscle fiber type. They also presented data to support that muscle fiber type parameters explain more variation in marbling scores than variation in tenderness. Ashmore (1974) reported that factors that promote large scale transformation of alpha-red fibers to alpha-white fibers may decrease marbling, thereby decrease quality grade. In addition, Melton et al. (1974) reported that red fiber area was significantly related to measures of increased animal weight and degree of fat deposition. They reported that cutability grade was highly correlated with red fiber area.

Hunt and Hendricks (1977) found that profile of fiber types of the *m. longissimus*, *m. psoas major*, *m. gluteus medius*, m. semitendinosus, and *m. semimembranosus* may provide insight to color stability. Hunt and Hendricks (1977) reported that the *m. psoas major* had the highest aerobic potential of the five muscles evaluated. They also found that the *m. semitendinosus* was dependent on location, as the inside *m. semitendinosus* had a higher aerobic potential than the outside *m. semitendinosus.* Atkinson and Follett (1973) stated that oxygen consumption rate is inversely correlated with rate of discoloration, thus when oxygen consumption rate is high there is increased deterioration of color pigments. This is directly related to muscle fiber types, as there is an increase in proportion of type I muscle fibers there will be an increase in mitochondria activity and

increase oxygen potential of the muscle (Klont et al. 1998). This increase in activity will in turn cause a faster onset of metmyoglobin formation.

Others

Enzymatic Reduction. The color of fresh meat is largely dependent on the relative proportions and distribution of the three pigments, deoxymyoglobin, oxymyoglobin, and metmyoglobin. Metmyoglobin is the least desirable, not only from the color standpoint, but also because ferric hemes act as catalyst in the oxidation of unsaturated lipids.

The proportion of myoglobin to oxymyoglobin is influenced by the activity of enzyme systems within the meat. The meat is capable of utilizing oxygen is evident from the simple observation that in oxygen-impermeable wrappings, surface oxymyoglobin quickly dissociates to myoglobin. Myoglobin may be also oxidized to metmyoglobin. The rate of autoxidation is dependent on oxygen tension and is highest at half saturation (George and Stratmann, 1952). The accumulation of metmyoglobin in stored meats is the resultant of these opposing factors (autoxidation and enzymatic reduction). The purpose of this research was to explore enzymatic pathways by which meat is able to reduce both oxygen and metmyoglobin, and the relation between oxygen and metmyoglobin reduction.

Upon slaughter, oxygen is cut off from the tissues and a rapid anaerobic glycolysis ensues. In postrigor meat, most of the glycogen has been converted to lactic acid, leaving a large pool of lactate. ATP and other high energy phosphates have disappeared.

If oxygen becomes available again, as when meat is ground or cut surfaces are exposed, the resumption of enzymic oxidase activity would be expected provided suitable hydrogen donors are present. Any succinate present should be rapidly oxidized by way of mitochondrial succinic dehydrogenase and electron-transport chain. This should result in a utilization of oxygen, but there is no known pathway for transfer of electrons form succinate to metmyoglobin (Watts et al., 1966)

USDA Quality Grades. Kennick and associates (1971) evaluated the effects of marbling and other variables on case life of New York Strip steaks. Degrees of marbling had a significant curvilinear effect on the case life of fresh steaks, with slight and slightly abundant amounts of marbling being the low point of the curve. These degrees of marbling are typical of USDA Good (Select) and Prime beef, respectively, In addition, Kennick et al. (1971) stated the three other degrees (small, modest and moderate) of marbling, which are typical of USDA Choice beef, and the most popular grade of beef sold, had longer case-life.

There have also been reports of differences in metmyoglobin formation among USDA quality grades. Correale et al. (1986) reported differences in metmyoglobin formation among strip steaks from three grades (Prime, Choice, Good) and attributed the differences to the inherent characteristics of the muscles. Muscle fibers from Prime and Choice samples were probably more red (as evidenced by greater marbling ability), while muscle fibers of Good (now Select) samples were probably more white (as evidenced by less marbling ability). This may be explained by higher cytochrome

activity by red muscles, which helps reduce metmyoglobin to myoglobin in the absence of oxygen.

Correale et al. (1986) examined differences among grades in different packaging techniques. They found grades had no significant effect ($P < 0.05$) on aerobic plate counts and did not result in major differences in the distribution of types of microflora of steaks. Surface discoloration and overall appearance scores of USDA Prime and Choice steaks packaged and stored in high oxygen barrier film often were more desirable ($P < 0.05$) than those of USDA Good (Select) steaks.

Lipid Oxidation. Many biochemical functions of meat work hand in hand when it comes to shelf-life. Lipid oxidation causes a rancid off-flavor and off odor in meat. Numerous factors affect lipid oxidation, e. g., light, oxygen concentration, temperature, presence of anti- and pro-oxidants, degree of unsaturation of the fatty acids, and presence of oxidative or reductive enzymes (Skibsted et al., 1998). Lipid oxidation is normally not considered to be a limiting factor for shelf-life or aerobic packed, chilled stored meat, as lipid oxidation occurs at a slower rate than discoloration or microbial growth (Zhao et al., 1994). However, when modified atmosphere packaging represses other deteriorative mechanisms in meat, lipid oxidation might become a major factor that limits meat shelf-life (McMillin, 1993). While an elevated oxygen level is known to prolong color stability, it nevertheless is also expected to increase the rate of lipid oxidation (Zhao et al., 1994). Increased lipid oxidation has been reported for meat stored at elevated oxygen concentrations (Jensen et al., 1997; Taylor, 1985), although other researchers did not find

an increase in lipid oxidation under similar conditions (Asenio et al., 1988; Lopez-Lorenzo et al., 1980; Ordonez and Ledward, 1977).

Ahn et al. (1998) evaluated the effect of muscle type, packaging, and irradiation on lipid oxidation. There was a slow increase in TBARS values from *m. longissimus dorsi* over a two week period. Under oxygen permeable packaging conditions TBARS values of patties from *m. longissimus dorsi* muscle increased by 10-fold over a two week storage period. Patties from *m. psoas* major and *m. rectus femoris* muscles had lower TBARS values than those from *m. longissimus dorsi* at all storage times. Increases of TBARS values in vacuum packaged *m. psoas major* and *m. rectus femoris* patties, as well as aerobic-packaged non-irradiated patties during the 14 day storage periods were small. Ahn et al. (1998) reported that total fat content of raw meat was an important factor closely related to the storage stability of meat.

Lawlor et al. (2000) evaluated the effect of muscle type and vitamin E treatments on lipid oxidation. They found that vitamin E decreased lipid oxidation over the 7 d of display. In addition, Lawlor et al. (2000) reported that the semitendinosus was the least stable compared to the *m. rhomboideus* or the *m. semimembranosus*.

O'Sullivan et al. (2002) found that steaks from cattle fed grass silage had higher alpha tocopherol levels than maize silage and maize silage:grass silage diets, whereby those with higher alpha-tocopherol levels had lower lipid oxidation and more sustainable color. Gatellier et al. (2001) evaluated vitamin E supplementation on color stability and found an increase in color shelf-life for those that were fed vitamin E. In addition, Gatellier et al. (2001) reported steaks packaged in MAP displayed much higher lipid

oxidation levels than those that were packaged in PVC. However, those controlled steaks packaged in MAP still had higher levels of lipid oxidation than treated steaks packaged in MAP. The study supports others that report that increasing oxygen in the packaging will increase lipid oxidation (Jensen et al., 1997).

O'Grady et al. (2001) reported that with increased lipid oxidation there is increased oxidation of myoglobin. However, they also reported that significant increases in lipid oxidation can occur before significant metmyoglobin is detected.

Residual Glycogen and pH. Muscle glycogen has also been targeted for a possible link to surface discoloration (Immonen et al., 2000). Muscle glycogen is the main metabolic substrate responsible for postmortem lactic acid accumulation and thus, normal pH decline. pH is a major role player in color stability as it affects many of the enzymatic activities in muscle. Bendall (1972) reported postmortem storage temperature and pH clearly affect mitochondrial activity as previously discussed. Bendall and Taylor (1972) found that as pH increased oxygen consumption rates increased. Immonen et al. (2000) reported about 45 mmol of glycogen is needed to lower the pH of 1 kg of muscle from 7.2 to 5.5. The concentration of glycogen varies greatly at the time of slaughter depending on the muscle, species, and nutritional status of the animal, but most of all on the level of pre-slaughter stress. The amount of lactic acid needed to produce a certain pH is quite constant, and therefore this pre-slaugheter range of glycogen is reflected to the residual glycogen concentration. Immonen and Puolanne (2000) found that at pH values below 5.75, bovine muscle residual glycogen concentration varied from 10 to 80 or more mmol/kg.

Inferior quality of meat of pH values above 5.8, commonly called dark-cutting, is well recognized. Its characteristics include reduced shelf-life (Gill and Newton 1981; Lawrie, 1958) and undesirably dark color (Lawrie, 1958). In addition to these, dark cutting has low or nonexistent surface glucose concentration (Gill and Newton, 1981).

The meat quality effects of residual glycogen have not been intensively studied. Residual glycogen may contribute to the shelf-life of meat to some extent, since during the early days post mortem the concentration of free glucose rises on the meat surface (Kress-Rogers et al., 1993), assumably because of hydrolysis and diffusion of residual glycogen (Kress-Rogers et al., 1993) and other fermentable substrates (Gill, 1996) from the deeper layers of the meat. Residual glycogen may also affect the flavor by enhancing the maillard browning reactions (Pethick et al., 1995).

Research on residual glycogen has mainly been done on pigs and pork quality. Monin and Sellier (1985) reported on the quality peculiarities concerning the Hampshire breed. According to their studies, the very low ultimate pH and high glycogen content caused low water holding capacity, low technological quality as well as the paleness of Hampshire pork. Differences in residual glycogen concentration and consequent differences in ultimate pork quality have later been proved to be genetic characteristics of the carriers of the dominant RN-allele (Estrade et al., 1993).

However, the potential effects of residual on the quality of beef have not been studied. This is surprising, because in beef, contrary to pork, the high concentrations of residual glycogen are likely to be accompanied with normal rather than extremely low pH values.

Immonen et al. (2000) reported that with increased glycogen levels there are decreases in redness (a*) values and increases in yellowness (b*) values. However, these differences were moderate numerically. Despite the moderate differences, this may play a role in overall color stability and just be another variable in overall color stability.

CHAPTER III

MATERIALS AND METHODS

Carcass Selection, Fabrication and Packaging

Beef carcasses (n = 18) at a commercial packing company were ribbed between the twelfth and thirteenth ribs for yield and quality grade data (USDA, 1997) following a 24-h chill period in a 1˚C cooler. Carcasses were selected to represent USDA Choice (n = 9) and USDA Select (n = 9). Six carcasses (n = 3) were selected on three different processing days. Nine muscles from each carcass were obtained to included three high color stability muscles (*m. longissimus lumborum*, *m. longissimus thoracis*, and *m. semitendinosus*), three medium color stability muscles (*m. semimembranosus*, *m. gluteus medius*, and *m. biceps femoris*), and three low color stability muscles (*m. supraspinatus, m. psoas major*, and *m. adductor*). All muscles were vacuum packaged (Cryovac®/Sealedair Corp, Ducan, SC) and stored at < 4˚C for a total of 10 d postmortem.

On d 10 postmortem, each muscle was cut into four 2.54 cm thick steaks and were allocated to one of 4 display days (1, 3, 5, and 7 d). Each steak was packaged in a Styrofoam tray and over-wrapped using an oxygen permeable polyvinyl chloride film (OTR = 6,500 cc/M^2, 24 h, atm, 25°C). Steaks were placed under retail display conditions for up to 7 display days. Retail display lights were suspended approximately 1 m above each display case and consisted of fluorescent natural white lights (Sylvania F40N, Osram Sylvania, Danvers, MA) and emitted a light intensity of 1200 lux.

Proximate Analysis

Proximate analysis was determined using a rapid determination of moisture and fat in meats by microwave and nuclear magnetic resonance analysis. Procedures were described by Keeton et al. (2003, AOAC; PVM1:2003). Approximately 4 grams of homogenized muscle were taken from a larger sample and analyzed for fat and moisture content. Moisture and fat was determined using a SMART Trac® system (microwave drying and NMR fat anlaysis system manufactured by CEM Corp.; Matthews, NC).

Bovine Heart Metmyoglobin Purification

Bovine heart metmyoglobin was extracted before each week of evaluation following procedures described by McKenna (2003) and subsequently used in the metmyoglobin reductase activity procedure. A 500 g sample of beef heart was taken, trimmed free of fat, epicardial and endocardial connective tissue. The sample was homogenized with 1000 mL of cold water using a Waring® blender for 60-90 sec. The pH was adjusted to 7.5 with 2N NH_4OH (Fisher Scientific, Fair Lawn, NJ). The sample then was centrifuged at 13,700 × g for 20 min (Avanti™ J-25, Beckman Coulter, Inc., Palo Alto, CA). The supernatant was removed and brought to 70% saturation with solid $(NH_4)_2SO_4$ (Fisher Scientific, Fair Lawn, NJ). It was adjusted to pH 7.5 with 2 N NH_4OH and stirred for 30 min. The sample was centrifuged at 13,700 × g for another 15 min. The solution was stirred for 30 min with 1 g of celite 545® (Fisher Scientific, Fair Lawn, NJ) added for every 100 mL. The sample then was vacuum filtered through glass wool (coarse porosity) (G/FD, Whatman International, Inc., Maidstone, UK) and the pink filtrate discarded. The red oxymyoglobin was eluted with 500-750 mL of cold distilled

water and centrifuged at 20,000 × g for 20 min. The supernatant then was filtered through glass wool. Excess $K_3Fe(CN)_6$ (Fisher Scientific, Fair Lawn, NJ) was added to oxidize the solution and then dialyzed 3 times (changing water each hour for the first 2 hrs and over night for the last dialysis step) using a 10,000 molecular weight cut-off dialysis tubing (Spectra/Pore, Spectrum Industries, Inc., Rancho Dominguez, CA) against distilled water. The sample was dialyzed against 2mM $NaPO_4$ buffer, pH 7.0. Then, the sample was concentrated to < 300 mL in an Amicon® Bioseparations Centricon® Plus-80 filters (Millipore Corp., Bedford, MA) and stored at 4°C.

Metmyoglobin Reductase Activity

The metmyoglobin reductase activity was measured as described by McKenna (2003) on each steak randomly allocated to a given evaluation day (1, 3, 5, and 7 d). A 4.45 cm diameter core was removed from each steak. The top 1/3 of each core was removed and finely chopped. Then, 5 g was homogenized with 25 mL of 2 mM phosphate buffer (pH 7.0) for 45 sec in a Waring® blender. Following blending, the homogenate was centrifuged at 35,000 x g for 30 min (4°C) and the supernatant was filtered through Whatman #541 filter paper. One or two crystals of $K_3Fe(CN)_6$ was added to oxidize the supernate and then it was poured into a 10,000 molecular weight cut-off dialysis tube (Spectra/Pore, Spectrum Industries, Inc., Rancho Dominquez, CA). The sample was dialyzed at 4°C against 2 mM phosphate buffer (pH 7.0) with two phosphate buffer changes. After dialysis, 0.2 mL of the muscle extract was placed in a micro-cuvette containing 0.1 mL of 5 mM disodium EDTA, 0.1 mL of 50 mM citrate buffer (pH 5.65), 0.1 mL of 3 mM potassium ferricyanide, 0.3 mL of 0.1 mM bovine

metmyoglobin, and 0.1 mL distilled-deionized water. The reaction was initiated by adding 0.1 mL of 10 mM NADH (β-Nicotinamide Adenine Dinucleotide, reduced form; Sigma-Aldrich Co., St. Louis, MO). The assay was run at 30°C and absorbance measured at 580 nm. Metmyoglobin reductase activity (nmoles/min·g) was calculated as described by McKenna (2003).

Myoglobin Content

Myoglobin content was determined on each evaluation day (1, 3, 5, and 7 d) using the muscle extract from the metmyoglobin reductase activity assay as described by McKenna (2003). One mL of muscle extract was placed in a micro-cuvette. Absorbance was read at 572, 565, 545, and 525 nm with a dual beam spectrophotometer (DU Series 7000, Beckman Instruments, Inc., Fullerton, CA). Myoglobin content was calculated as described by Kryzwicki (1979). The following formula was used to determine myoglobin content: $(-0.166*R_1 +0.86*R_2 + 0.088*R_3 + 0.099)*R_4$, where R_1, R_2, R_3, and R_4 = absorbance ratios of A^{572}/A^{525}, A^{565}/A^{525}, A^{565}/A^{545}, and A^{545}, respectively.

Metmyoglobin Content

Using the procedure of Kryzwicki (1982), the relative metmyoglobin content was determined on 1, 3, 5, and 7 d. A 40 mM phosphate buffer solution with a pH of 6.8 was used. A 4.0 g meat sample from the same steaks was blended in a Waring® Commercial blender with 36 mL of the 40 mM phosphate buffer. The homogenate was placed into a 50 mL centrifuge tube and suspended in ice for 2 h to extract the pigment. After 2 h, the tubes were centrifuged for 30 minutes at 30,000 g using an Avanti™ J-25 Centrifuge (Beckman Coulter™, U.S.) at 5°C. The supernatant was filtered through Whatman No. 1

filter paper and absorbance of the solution read at 525, 545, 565, and 572 nm with a dual beam spectrophotometer (DU Series 7000, Beckman Instruments, Inc., Fullerton, CA) and recorded. Exposure to light was avoided throughout the procedure to reduce further oxidation of the pigment. The following formula was used to calculate metmyoglobin content. Metmyoglobin content (mg/g) = $-2.514\ R_1 + 0.777\ R_2 + 0.800\ R_3 + 1.098$. Where R_1, R_2, and R_3 = absorbance ratios of A^{572}/A^{525}, A^{565}/A^{525}, and A^{545}/A^{525}, respectively.

Aerobic Reducing Ability

Aerobic reducing ability was determined on each evaluation day (1, 3, 5, and 7 d) as described by McKenna (2003). A 4.45 cm core was removed from each steak, placed on a mini-Petri dish (47 mm, Millipore Corp., Bedfored, MA), and the sample was wrapped with an oxygen permeable film (D14, Shield Manufacturing Corporation, Oklahoma City, OK) to prevent surface dehydration. The muscle sample was placed in a 1% O_2/ 99% N_2 environment for 24 h at < 4°C. A Hunter Mini Scan XE (HunterLabs, Reston, VA) with a 1.27-cm aperture, 10° standard observer, and light source A was standardized using a white tile and used to measure the reflectance at 400 – 700 nm. Following the initial 24 h storage period, the sample was stored for an additional 24 h in atmospheric O_2 and reflectance was measured as described above. Percentage of metmyoglobin was calculated following AMSA (2003) guidelines. Aerobic reducing ability (ARA) was determined as described by McKenna (2003) using the following formula: ARA = (Decrease in MetMb / Initial MetMb) × 100.

Oxygen Consumption Rate

Polypropylene bottles (250 mL) with caps that had been modified to include a septum were used to collect oxygen consumption data. A 4.45 cm diameter core was removed from each steak and placed into the polypropylene bottles. Polypropylene bottles were flushed with oxygen and the initial oxygen and carbon dioxide concentrations in each bottle were measured using a headspace analyzer (PBI Dansensor Checkpoint, PBI-Dansensor A/S, Ringted, Denmark). Polypropylene bottles were stored at 4°C for 12 h and the final O_2 and CO_2 concentrations were measured. Oxygen consumption was determined by subtracting the initial carbon dioxide concentration from the final carbon dioxide concentration as described by McKenna (2003).

Oxygen Penetration Depth

Determination of oxygen penetration depth followed procedures described by McKenna (2003). Two, 5 × 5 cm muscel tissue slices approximately 1 cm thick from each muscle were taken transversely to expose an anaerobic portion. The muscle tissue was immediately placed between two 5.1 cm × 5.1 cm glass plates, with the anaerobic portions of the cut placed directly against the plate. The glass plates were taped together and over-wrapped with polyvinyl chloride film (PVC) to prevent moisture loss. The plates were displayed under normal retail display conditions as previously described. Five measurements of the depth of the oxymyoglobin layer were taken using a digital caliper (Control Company, Friendswood, TX) and averaged once per evaluation day.

Colorimeter Evaluation

A reflectance was measured over the range of 400 – 700 nm using a Hunter Mini

Scan XE (Hunter Labs, Reston, VA) with a 1.27 cm aperture, 10° standard observer and light source A to examine CIE L*, a*, and b* color space values on 1, 3, 5, and 7 d. Each package was evaluated at 2 locations on the cut surface and averaged.

pH

pH of each steak was measured on each evaluation day (1, 3, 5, and 7 d) using a handheld pH meter (pHStar, SFK Technologies, Denmark). Two measurements were taken from each steak and averaged.

2-Thiobarbituric Acid Reactive Substances

Beef steaks were analyzed in duplicate for 2-thiobarbituric acid reactive substances (TBARS), as described by Witte et al. (1970), on each evaluation day (1, 3, 5, and 7 d) to provide a measure of lipid oxidation within each treatment. The procedure included the following reagents. Thiobarbituric acid (TBA) solution was made by combining 10 g TBA and 1 g NaOH, dissolved in 1 L of distilled water. The trichloroacetic acid-HCL reagent was made with 25 g TCA and 6 mL 0.6 N HCl, brought to volume with 1 L of distilled water. The antioxidant solution was made by mixing together 0.6 g BHA in 10.8 g propylene glycol and 0.6 g BHT in 8.0 g warm Tween 20.

A 0.2 to 0.4 g portion of the ground sample was weighed, placed in a 25 mL screw cap test tube, and the exact weight recorded. Three drops of the antioxidant solution and 3 mL of TBA solution were added to each sample, and the sample shaken to disperse the meat. Then, 17 mL of TCA-HCl solution was added and the sample shaken again. The test tubes were flushed with N_2, tightly sealed, heated in a boiling water vat for 30 minutes and allowed to cool. Then, 2.5 mL of the solution was transferred to a test

tube, and 2.5 mL of chloroform added. The sample was centrifuged using a Jouan C4-12 Centrifuge (Jouan, Inc., U.S., Model # C4-12) for 5 minutes at 3000 rpm. The upper phase was transferred into a plastic micro-cuvette and the absorbance read at 532 nm using a spectrophotometer (DU Series 7000, Beckman Instruments, Inc., Fullerton, CA). A blank was run each sample day and samples analyzed in duplicate. The following formula was used to calculate lipid oxidation in mg/g.

Mg malonaldehyde/kg = [(abs sample-abs blank) × 4.6]/g sample.

Color Panel

All steaks were visually evaluated before removal from the package on days 1, 3, 5 and 7 by a panel consisting of trained A&M personnel (n=6). The panel was trained according to AMSA (1995) guidelines. Each steak was evaluated for lean color and assigned a score between 8 (bright cherry-red) to 1 (extremely dark red) and surface discoloration with scores ranging from 1 (0%) to 7 (100% discoloration) (Appendix I, Figure 1; AMSA, 1995). Panelists were given color blocks before each day's evaluation (Appendix I, Figure 2).

Statistical Analysis

All data except percent fat and moisture were analyzed utilizing the general linear model (GLM) procedure of SAS (version 9.0, SAS Institute, Cary, NC). Data were analyzed as a split-split-plot and carcass was defined as an experimental unit. Grade was defined as the main effect in the whole-plot and carcass(grade) was used as the whole-plot error term. In the first split, muscle and muscle × grade were defined as effects. The error for the first split was muscle by carcass(grade). The second split included storage

day, storage day by grade and storage day by muscle effectrs. Residual error was used to test these effects. Significance was predetermined at $\alpha < 0.05$. If interaction effects were not significant, they were pooled into the residual error. For main and interaction effect that were significant, least squares means were calculated and differences between means were determined using pdiff function. Percent fat and moisture were analyzed in a completely randomized block design utilizing the general linear model (GLM) procedure of SAS. When main effects were determined to be significant ($P < 0.05$), least squares means were generated and separated using a pairwise t test (PDIFF option). All possible pair-wise comparisons were made with a significance level of 5% for the response variables (SAS Institute, Inc., Cary, NC).

CHAPTER IV

RESULTS AND DISCUSSION

Many consumers evaluate steaks by the color in making their purchasing decision and beef steak quality is equated to a bright red color. Consumers look unfavorably towards discoloration and variation from the bright red standard. The current study evaluated nine bovine muscles from Select and Choice carcasses. Data showed Select steaks scored higher than Choice steaks by the panelists, while other studies have reported opposite trends (Kennick et al., 1971; Behrends et al., 2003). Kennick et al. (1971) reported color desirability was higher for those in the Choice grades then those in the Select and Prime grades.

There continues to be differences between muscles when color is evaluated over display time. The evaluation of the nine muscles in the current study yielded some insight into possible differences between muscles, while much work remains to be done in the field of fresh meat color stability. This study presents data that supports and differs from previous research.

The color of fresh meat is largely dependent on the relative proportions and distribution of the three pigments, deoxymyoglobin, oxymyoglobin, and metmyoglboin. Metmyoglobin is the least desirable, not only from the color standpoint, but also because ferric hemes act as catalyst in the oxidation of unsaturated lipids. This can be seen by the current findings by the increase in lipid oxidation over retail display, which in turn acted as an accelerator for increased metmyoglobin formation in all muscles especially those with low color stability (e.g., *m. psoas major*).

Proximate Analysis

Percent fat and moisture were evaluated to assess differences between muscles and grades. There was a muscle × grade interaction for percent fat (Table 1). The *m. gluteobiceps*, *m. gluteus medius*, and *m. semitendinosus* from the Choice carcasses tended to be higher in percent fat than those from Select carcasses. The *m. longissimus lumborum*, *m. longissimus thoracis*, and *m. psoas major* from Choice carcasses were significantly different from those from the Select carcasses, whereas the *m. adductor* and *m. supraspinatus* were not different. Higher percent fat contributes to increased levels of lipid oxidation. Also, lipid oxidation may cause increased metmyoglobin formation, which contributes to color deterioration.

Table 1. Least squares means for percent fat for muscle × grade interactions ($P = 0.0237$)

	GRADE	
Muscle	Choice	Select
AD[h]	2.16[g]	2.15[g]
GB[h]	4.80[ab]	4.14[bc]
GM[h]	3.46[c-e]	2.88[d-g]
LL[h]	4.99[ab]	3.00[d-g]
LT[h]	4.87[ab]	3.27[c-f]
PM[h]	5.44[a]	4.14[bc]
SM[h]	2.53[fg]	2.46[fg]
SS[h]	3.54[cd]	3.30[c-f]
ST[h]	3.51[c-e]	2.61[e-g]

[a-g]Means for percent fat without a common superscript letter differ ($P < 0.05$).
[h]AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*.
*SEM is the standard error of least squares means (SEM = 0.322)

Muscles were different for moisture ($P < 0.0001$; Table 2). The *m. semitendinosus*, *m. semimembranosus*, and *m. adductor* had the highest percent moisture while the *m. longissimus lumborum*, *m. longissimus thoracis*, and *m. psoas major*, had the lowest percent moisture. Grades were also different ($P < 0.0001$) in moisture content, as Select was higher in percent moisture than Choice (73.13 versus 73.92, respectively).

Table 2. Least squares means for percent moisture for muscle ($P = < 0.0001$)

MUSCLE	%MO[e]
m. adductor	73.90[a]
m. gluteobiceps	73.02[cd]
m. gluteus medius	73.50[c]
m. longissimus lumborum	72.27[d]
m. longissimus thoracis	72.82[cd]
m. psoas major	72.94[cd]
m. semimembranosus	73.86[a]
m. supraspinatus	73.67[b]
m. semitendinosus	74.05[a]
SEM*	0.274

[a-d]Means within the same column without a common superscript letter differ ($P < 0.05$)
[e]%MO = percent moisture. *SEM is the standard error of least squares means

Metmyoglobin

Those muscles which are considered high color stability (i.e., *m. longissimus thoracis* and *m. longissimus lumborum*) had the lowest percent metmyoglobin (Table 3). Other muscles which are intermediate and low color stability had much higher percent

metmyoglobin (i.e., *m. gluteobiceps*, *m. gluteus medius*, and *m. psoas major*). All muscles increased in percent metmyoglobin during display. On d 5 percent metmyoglobin reached unacceptable levels (Table 4). Grade was not significant ($P > 0.05$) for percent metmyoglobin. However, others have reported differences in metmyoglobin between grades.

Table 3. Main effects of muscle on percent metmyoglobin ($P < 0.0001$)

MUSCLE	%Metmb[f]
m. adductor	20.64[bcd]
m. gluteobiceps	29.23[a]
m. gluteus medius	22.83[bc]
m. longissimus lumborum	14.62[e]
m. longissimus thoracis	13.45[e]
m. psoas major	24.79[ab]
m. semimembranosus	18.66[cde]
m. supraspinatus	17.08[de]
m. semitendinosus	17.58[cde]
SEM*	1.974

[a-e]Means within the same column without a common superscript letter differ ($P < 0.05$)
[f]%Metmb = percent metmyoglobin
*SEM is the standard error of least squares means

Correale et al. (1986) reported differences in metmyoglobin formation among strip steaks from the three grades (Prime, Choice and Good) and attributed the differences to the inherent characteristics of the muscles. Muscle fibers from Prime and Choice samples were probably more red (as evidenced by greater marbling ability),

while muscle fibers of Good (now Select) samples were probably more white (as evidenced by lesser marbling ability). Correale et al. (1986) suggested that a possible increase in cytochrome activity by red muscles may help reduce metmyoglobin to myoglobin in the absence of oxygen. The *m. adductor* was reported to have a high amount of alpha-white fibers by Kirchofer et al. (2002). This may explain why those muscles in the present study with poor color stability, such as the *m. adductor*, exhibited high amounts of metmyoglobin. Ledward (1971) found that the *m. biceps femoris* (*m. gluteobiceps*) and *m. semimembranosus* had a higher metmyoglobin concentration than the *m. longissimus dorsi*, which supports the present study.

Table 4. Main effects of day on percent metmyoglobin ($P < 0.0001$)

	Day				
	1	3	5	7	SEM*
%Metmb[d]	2.06[a]	14.20[b]	30.40[c]	32.75[c]	1.375

[a-c]Means within the same column without a common superscript letter differ ($P < 0.05$)
[d]%Metmb = percent metmyoglobin
*SEM is the standard error of least squares means

Renerre and Labas (1987) reported that consumers begin to discriminate against steaks when approximately 20% metmyoglobin is present. The current study showed levels of 15% or more by d 3 for low color stability muscles (e.g. *m. psoas major*, *m. adductor*, and *m. gluteobiceps*). Previous research has characterized the *m. psoas major* as having very low color stability and *m. longissimus lumborum* and *m. longissimus thoracis* as having very high color stability (Echevarre et al., 1990; Renerre and Labas,

1987), which supports the current findings of this study. As found in the current study, Bendall and Taylor (1972) found that the *m. biceps femoris* (*m. gluteobiceps*) had relatively unstable color. Faustman and Cassens (1991) reported that the *m. longissimus* displayed a greater percentage of metmyoglobin than the *m. gluteus medius*, which agrees with the current findings supporting the evidence that the *m. longissimus* is a much more color stable muscle.

Myoglobin

There was a muscle × day interaction for myoglobin content (Figure 5). Muscles reacted differently over retail display. Myoglobin content did not change during the first 3 d of display for the *m. gluteus medius*, *m. longissimus lumborum*, *m. longissimus thoracis*, and *m. semimembranosus*; however, a decline was observed for the previously mentioned muscles during the last two days of retail display. All muscles displayed lower myoglobin content on d 7 of display than on d 1. The *m. semitendinosus* displayed the least change during retail display (Figure 5). Myoglobin was higher in Choice than in Select steaks (4.68 versus 4.25, respectively), as higher marbled beef has a higher myoglobin content, as well as increased mitochondrial activity and oxidative potential.

Myoglobin content decreased over time in the present study, which supports the findings by McKenna (2003). It is believed that during display there is degradation of myoglobin, in turn causing the decrease in myoglobin content between muscles. Furthermore, with the high amount of oxidation between many of the low color stable muscles, it can be assumed that loss of recoverable myoglobin would exist. MacDougall

and Taylor (1975) and Reddy and Carpenter (1991) found less myoglobin in *m. psoas major* than in the *m. longissimus lumborum et thoracis*, which agrees with the current findings of this study. However, on d 7 of this study the *m. longissimus thoracis* had no difference when compared to the *m. psoas major*.

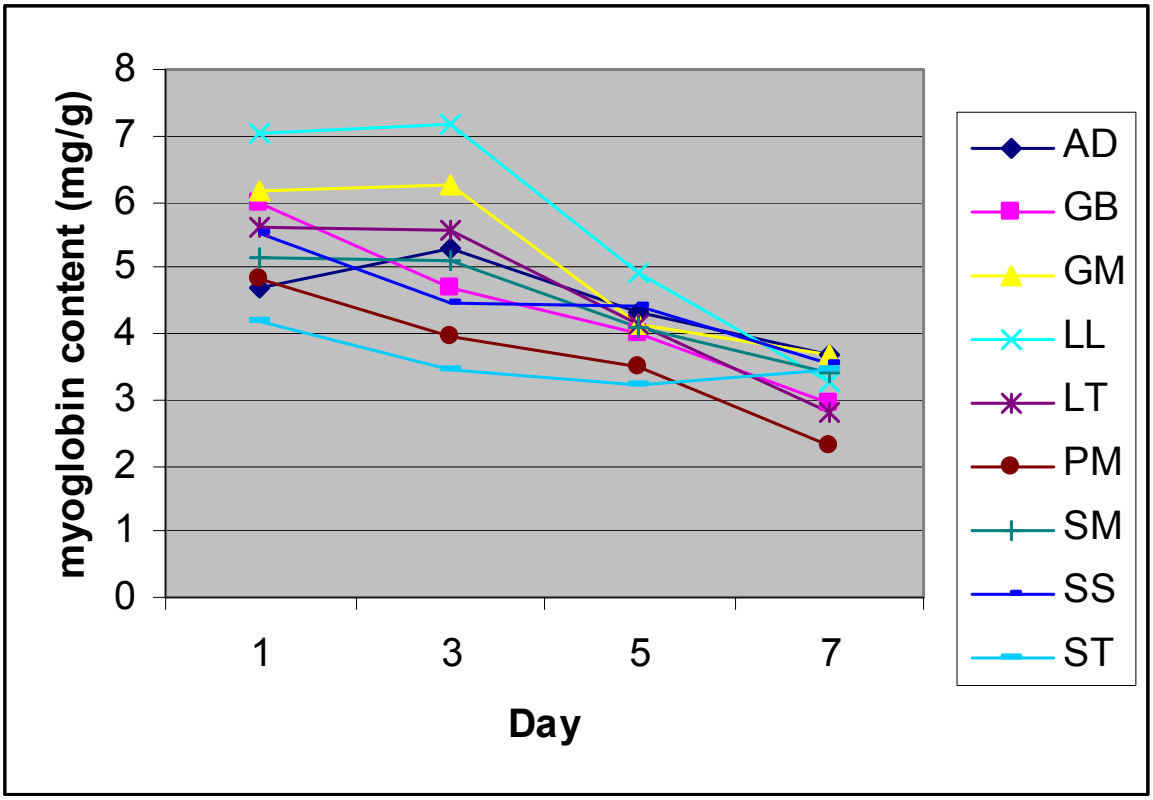

Figure 5. Least squares means for myoglobin content (mg/g) for muscle × day interactions ($P = 0.0032$). AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*. *SEM is the standard error of least squares means (SEM = 0.435)

TBARS

Few differences were found between muscles, as those muscles with low color stability (i.e., *m. adductor* and *m. psoas major*) had much higher TBARS values than high color stability muscles (i.e., *m. longissimus thoracis* and *m. longissimus lumborum* (Table 5). The day × grade interaction ($P < 0.05$) showed steaks from Choice and Select did not differ on 1, 3, and 5 d of retail display; however, on d 7 Choice steaks displayed a significantly higher amount of lipid oxidation versus Select steaks (Figure 6). Also, both Choice and Select steaks increased dramatically from d 5 to d 7.

Table 5. Main effects of muscle on TBARS ($P < 0.0142$)

MUSCLE	TBARS[f]
m. adductor	0.28[a]
m. gluteobiceps	0.21[b]
m. gluteus medius	0.27[a]
m. longissimus lumborum	0.20[b]
m. longissimus thoracis	0.21[b]
m. psoas major	0.28[a]
m. semimembranosus	0.26[ab]
m. supraspinatus	0.23[ab]
m. semitendinosus	0.27[a]
SEM*	0.028

[ab]Means within the same column without a common superscript letter differ ($P < 0.05$)
[f]TBARS – 2-thiobarbituric acid reactive substances (mg malonaldehyde/kg)
*SEM is the standard error of least squares means

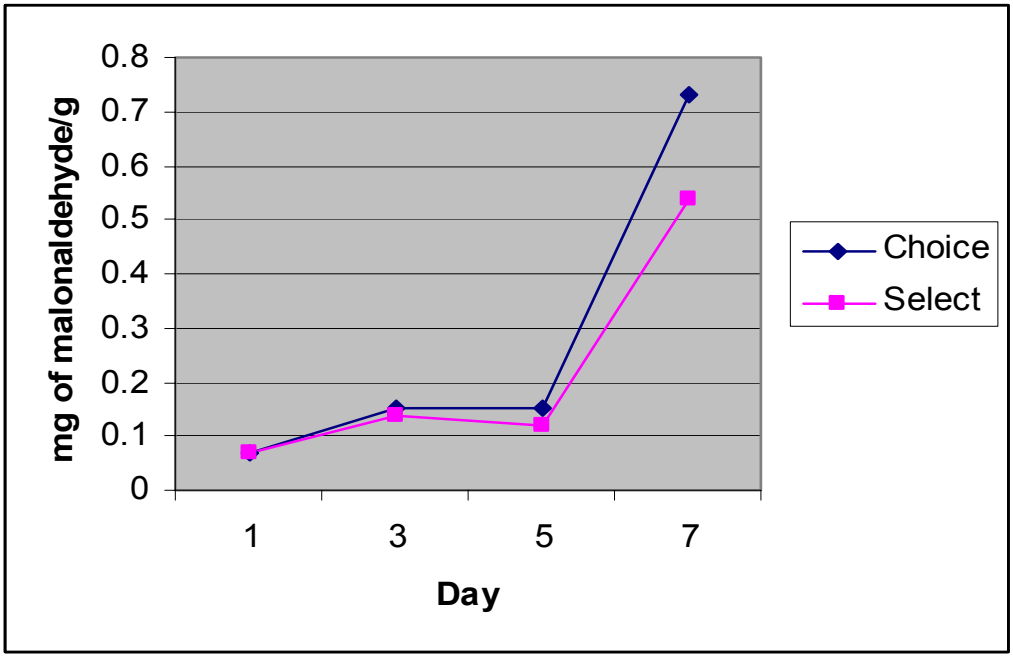

Figure 6. Least squares means for TBARS (mg/kg) for day × grade interactions ($P <$ 0.0054). *SEM is the standard error of least squares means (SEM = 0.029)

Lipid oxidation continues to play a major role in retail display shelf-life. The current study showed that increase in lipid oxidation occurred in those with higher amounts of fat content. The TBARS values were highly correlated with discoloration during retail display, which agrees with Faustman and Cassens (1990) who found increases in lipid oxidation caused an increase in oxidation of the pigments. These findings suggest that as lipids are oxidized, free radicals are produced, which cause a destruction of the pigments, thereby causing increased discoloration and metmyoglobin formation.

Metmyoglobin was shown to accelerate in the presence of oxidizing the lipids, although this may only occur if there are large increases in lipid oxidation (O'Grady et al., 2001). O'Grady and others (2001) reported that lipid oxidation does not by itself

promote metmyoglobin; however, primary lipid peroxide products or free-radical breakdown products in the presence of oxymyoglobin may cause the formation of metmyoglobin. As shown in the current study, when lipid oxidation increased, metmyoglobin increased.

Metmyoglobin Reductase Activity

Differences between muscles were found for metmyoglobin reductase activity. The *m. adductor* displayed the highest metmyoglobin reductase activity, while the *m. semitendinosu*s displayed the lowest values (Table 6). The *m. adductor*, *m. gluteobiceps*, *m. gluteus medius*, *m. longissimus lumborum*, *m. longissimus thoracis*, *m. psoas major* and *m. supraspinatus* all had high metmyoglobin reductase activity. Metmyoglobin reductase activity over time showed no real trends; however, there were differences between days (Table 7).

Table 6. Main effects of muscle on metmyoglobin reductase activity ($P < 0.0001$)

MUSCLE	MRA[e]
m. adductor	285.34[a]
m. gluteobiceps	266.40[bc]
m. gluteus medius	277.27[ab]
m. longissimus lumborum	260.10[c]
m. longissimus thoracis	261.75[bc]
m. psoas major	270.65[abc]
m. semimembranosus	269.76[abc]
m. supraspinatus	284.69[a]
m. semitendinosus	220.40[d]
SEM*	5.667

[a-d]Means within the same column without a common superscript letter differ ($P < 0.05$)
[e]MRA = metmyoglobin reductase activity (nmoles/min·g)
*SEM is the standard error of least squares means

Table 7. Main effects of day on metmyoglobin reductase activity ($P < 0.0001$)

	Day				
	1	3	5	7	SEM
MRA[d]	288.62[a]	235.50[c]	277.64[a]	263.59[b]	4.291

[a-c]Means within the same column without a common superscript letter differ ($P < 0.05$)
[d]MRA = metmyoglobin reductase activity (nmoles/min·g)
*SEM is the standard error of least squares means

Sammel et al. (2002) reported direct measurement of the reductase enzymes does not correlate with color stability, which helps to support the present study. In addition, it is possible that addition of NADH *in vitro* does not accurately represent the actual metmyoglobin reducing environment. This may help to explain the inaccurate results found in the current study, which found those muscles of low color stability, such as *m. adductor, M. gluteus medius, m. gluteobiceps, m. semimembranosus, and m. supraspinatus* displaying high metmyoglobin reductase activity. The *m. semitendinosus* tends to be a high color stability muscle and possess low levels of metmyoglobin reductase activity. McKenna (2003) found that those muscles with low color stability generally had the highest metmyoglobin reductase activity, whereas the *m. semitendinosus,* a more color stable muscle, had lower metmyoglobin reductase activity, which supports the current study. Findings from McKenna (2003) support the current data that the *m. longissimus* had high metmyoglobin reductase activity. As was found in the present study, Renerre and Labas (1987) reported no differences in metmyoglobin reductase activity between a low color stability muscle, such as the *m. psoas major,* and a high color stability muscle, such as the *m. tensor fasciae latae*. Also, Reddy and Carpenter (1991) reported that metmyoglobin reductase activity for muscles was higher

in *m. tensor fasciae latae* > *m. longissimus lumborum* > *m. gluteus medius* > *m. semimembranosus* = *m. psoas major*, which conflicts with this study that reports no differences between low and high color stability muscles.

The present studies findings conflict with earlier work, which states that low color stability muscles have a lower metmyoglobin reducing ability (O'Keeffe and Hood, 1982); however, this was not a measure of specific reductase activity. Madhavi and Carpenter (1993) reported that *m. psoas major* steaks had greater metmyoglobin accumulation and lower metmyoglobin reductase activity than the *m. longissimus*. Hutchins et al. (1967) reported that as metmyoglobin reductase activity increases, there is a tendency for less metmyoglobin formation. Others have supported the fact that there is little relationship of metmyoglobin reductase activity to discoloration in beef, pork and lamb (Atkinson and Follett, 1973).

Recent studies have shown that metmyoglobin reductase activity is not accurate in assessing color stability of different muscles (McKenna, 2003; Renerre and Labas, 1987). Ledward (1972) reported a lack of correlation between the metmyoglobin reductase activity and metmyoglobin formation. The current study showed few differences between muscles, in addition to inconsistent results. It seems that metmyoglobin reductase activity is highly dependent on NADH, the rate limiting substance. Furthermore, addition of ferricyanide, which is added to the sample, may act as an interference with the reducing reaction. The results by many suggest either the technique for metmyoglobin reductase activity estimation does not accurately measure

the activity of the enzymatic reduction or that the effective aerobic deduction does not occur by this mechanism.

Aerobic Reducing Ability

Aerobic reducing ability displayed a muscle × day interaction, as the highest activity was recorded on d 1 of the retail display for all muscles except *m. longissimus lumborum* and *m. longissimus thoracis* (Figure 7). Muscles lost aerobic reducing ability very quickly during retail display; however, the *m. longissimus lumborum* and *m. longissimus thoracis* showed a curvilinear effect over retail display, peaking on d 5. Muscles, such as *m. adductor*, *m. psoas major*, *m. semimembranosus*, and *m. supraspinatus*, that tend to have poor color stability, had the highest initial aerobic reducing ability; however, lost this ability by day 3 of retail display (Figure 7). Choice steaks displayed higher reducing ability when compared to select steaks (3.34 versus 2.10 %Δ metmyoglobin, respectively).

Sammel et al. (2002) also evaluated different methods to assess color stability and found that metmyoglobin reductase activity was not an accurate measurement of color stability. However, Sammel et al. (2002) reported that aerobic reducing ability correlated best with color stability over retail display, which agrees with some of the present correlations found in the current study. Renerre and Labas (1987) stated the high tissue oxygen consumption in meat discoloration process is an important factor in color stability and that metmyoglobin reductase activity in different muscles in not an accurate measurement of color stability. Ledward (1985) agreed with the findings that oxygen consumption is important and maintains that enzymatic reducing activity is the

most important factor to determine metmyoglobin accumulation. The current findings agree that oxygen consumption plays some role in color stability; however, there is some evidence to support enzymatic reducing activity and its effect on meat color stability, despite the current accuracy of the measurements available. It is still too difficult to fully understand the mechanistic effects of enzymatic reduction in meat and continued evaluation of new procedures to more properly identify metmyoglobin reducing activity is essential to help fully understand its effect on color stability.

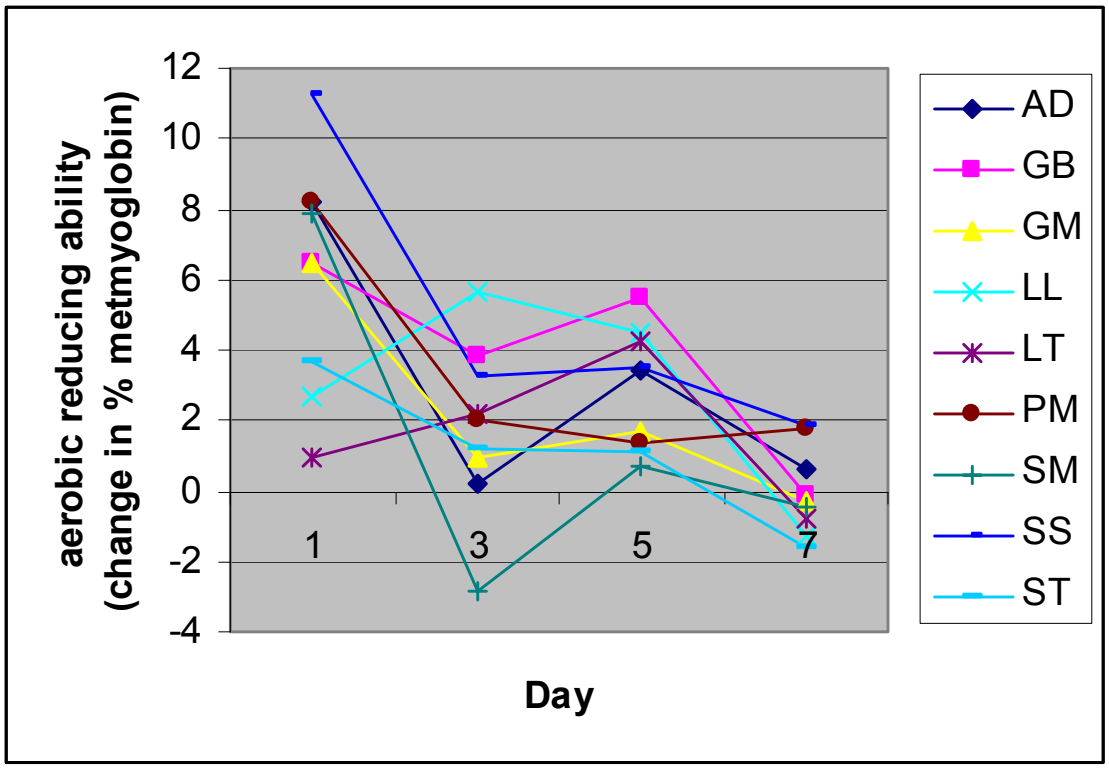

Figure 7. Least squares means for aerobic reducing ability (Δ% metmyoglobin) for muscle × day interactions ($P < 0.0024$). AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus.* *SEM is the standard error of least squares means (SEM = 1.195)

As shown in the present study, muscles tended to differ in aerobic reducing abilities, which was also reported by O'Keeffe and Hood (1982). The current study found those muscles with low color stability (e.g., *m. psoas* major and *m. adductor*) had initially high aerobic reducing ability, however, after d 1 of display quickly decreases. Much of this decrease of aerobic reducing ability stems from those muscle of low color stability reaching high levels of metmyoglobin early in the retail display period. O'Keeffe and Hood (1982) found very high correlation of aerobic reducing ability and discoloration, whereas the present study found a significace, but low correlation. Sammel et al. (2002) and Faustman and Cassens (1990) believe that aerobic reducing ability is a good measurement because no chemicals are used as oxidizing agent, thus the inherent reducing system is only minimally altered.

Oxygen Consumption Rate and Oxygen Penetration Depth

There were no differences between muscles for oxygen consumption rate ($P > 0.05$). Moreover, no differences were evaluated between grades for oxygen consumption rate ($P > 0.05$). However, the highest oxygen consumption rate was evaluated on d 1, but very little oxygen consumption rate was seen on any of the other retail display days (Table 8).

The current study found very few differences due to oxygen consumption rate. Despite these findings, others have found that oxygen consumption rate plays a major role in oxidative and enzymatic activity. Renerre and Labas (1987) reported that muscles that have low color stability have the highest oxidative activity. Further research by Sammel et al. (2002) indicated that very high and very low oxygen

consumption rates had a negative impact on overall color stability. High oxygen consumption is predicted to have an affect on partial oxygen pressure, which could result in a thin oxymyoglobin layer. The low oxygen consumption may account for low mitochondrial activity, thereby reducing NADH, a rate limiting step in reducing activity. These findings can not be supported by the present study because there was a lack of consistency in oxygen consumption of the same muscles possibly due to the modified procedure.

Table 8. Main effects of day on oxygen consumption rate (%Δ CO_2) ($P < 0.0001$)

	Day				
	1	3	5	7	SEM*
OCR[d]	1.42[a]	0.68[b]	0.43[c]	0.52[bc]	0.064

[a-c]Means without a common superscript letter differ ($P < 0.05$)
[d]OCR = oxygen consumption rate (Δ% CO_2)
*SEM is the standard error of least squares means

In addition, the current study did see some reducing ability and oxygen consumption at d 1, but this quickly fell in most muscles during retail display. Initially, those muscles that contained high oxygen consumption rates (e.g., *m. adductor*) showed the largest decrease in oxygen consumption rate, as well as the most discoloration over time and lowest color stability as in agreement with Renerre and Labas (1987).
There was a muscle × day interaction for oxygen penetration depth (Figure 8). There was a curvilinear effect of each muscle over time. The *m. longissimus lumborum* and *m. longissimus thoracis* displayed the highest oxygen penetration depth throughout the

retail display. The *m. adductor*, *m. gluteobiceps*, *m. gluteus medius*, *m. psoas major*, *m. semimembranosus* and *m. supraspinatus* displayed the least change and lowest penetration over retail display time (Figure 8).

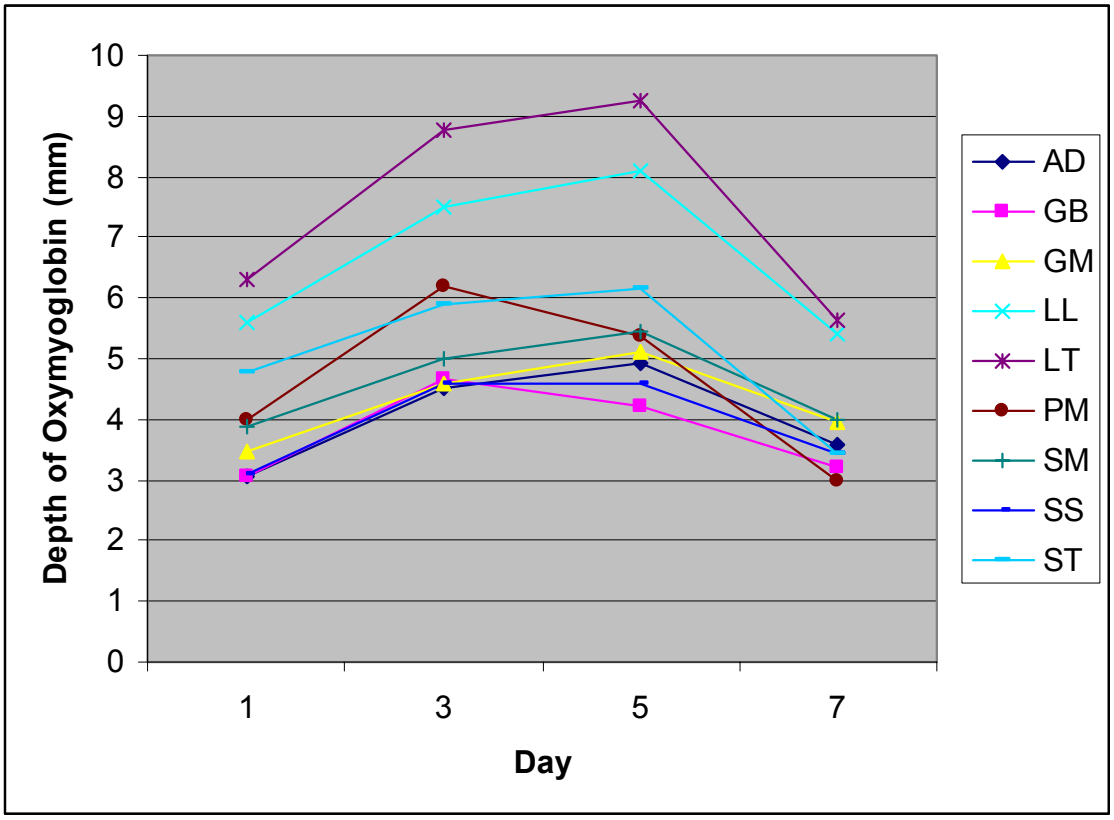

Figure 8. Least squares means for oxygen penetration depth (mm) values for muscle × day interactions ($P < 0.0001$). AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus.* *SEM is the standard error of least squares means (SEM = 0.309)

The *m. psoas major* and *m. gluteobiceps* had the lowest oxygen penetration depth on day 7 of the retail display. Muscle × grade interaction was significant, whereas the *m. gluteobiceps*, *m. longissimus thoracis* and *m. psoas major* had greater oxygen

penetration depth in Select samples versus Choice samples (Figure 9). However, the *m. adductor*, *m. gluteus medius*, *m. semimembranosus*, and *m. supraspinatus* tended to have a lower oxygen penetration depth in Choice samples versus their Select counterparts.

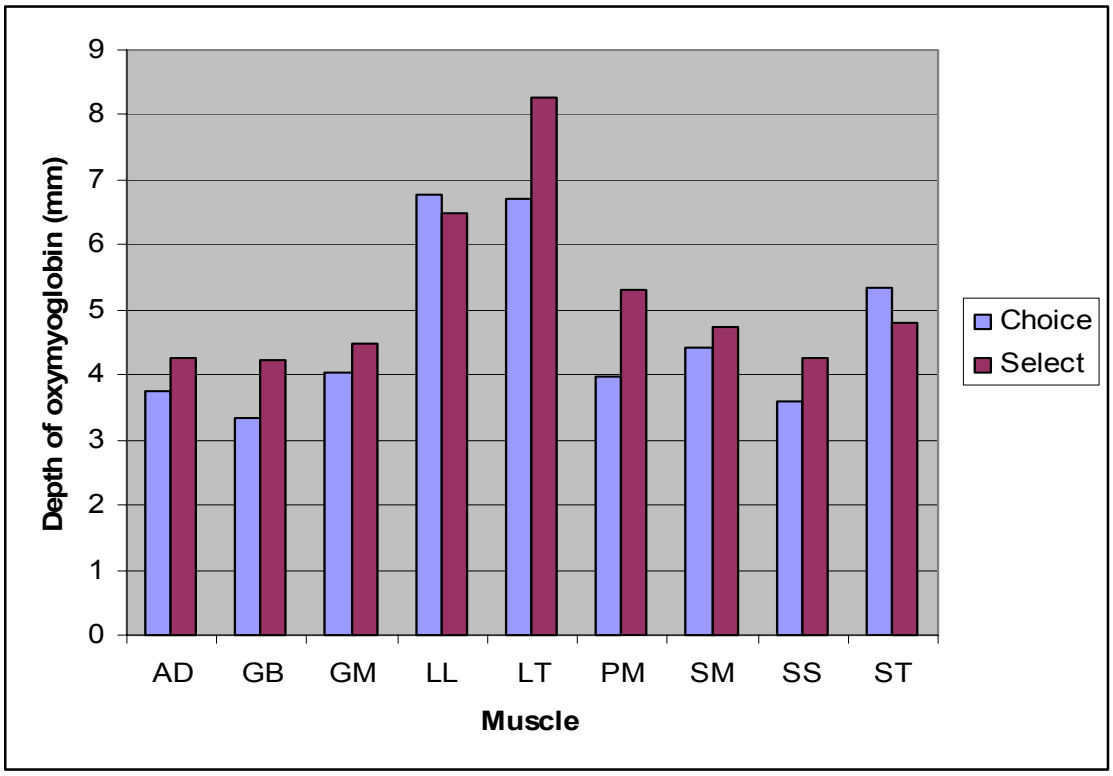

Figure 9. Least squares means for oxygen penetration depth (mm) values for muscle × grade interactions ($P = 0.0424$). AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus.* *SEM is the standard error of least squares means (SEM = 0.334)

There was a day × grade effect for oxygen penetration depth, as shown by Select samples having a greater oxygen penetration depth over the retail display period versus Choice samples (Figure 10).

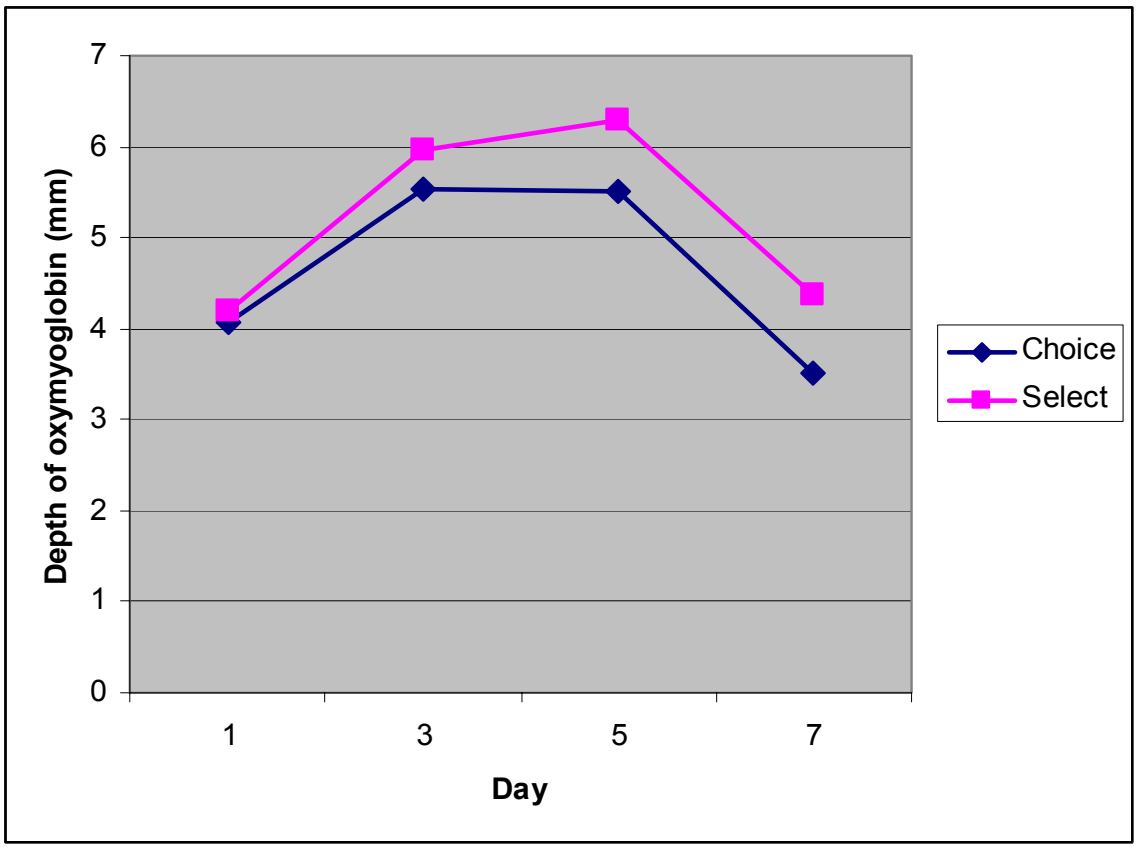

Figure 10. Least squares means for oxygen penetration depth (mm) values for day × grade interactions ($P = 0.0498$). *SEM is the standard error of least squares means (SEM = 0.0146)

Oxygen consumption rate is associated with residual mitochondrial respiration in postmortem muscle (Bendall and Taylor, 1972) and is very difficult to measure without proper equipment. The current study utilized modified polypropylene bottles. Despite a very small change in CO_2 in the bottles, there were still trends between muscles and display time. The identification of low color stability muscles, such as the *m. adductor* and *m. psoas major*, could possibly be identified by their oxygen consumption rate which fell dramatically by d 3 of retail display. It seems that muscle quickly utilizes oxygen in the first 3 d of retail display, possibly explaining the low CO_2 levels found on

3, 5, and 7 d of retail display. The present study presents data that oxygen consumption rate and oxygen penetration depth are negatively correlated, with oxygen penetration depths increasing as oxygen consumption rate decreases. However, this correlation was very low. Despite these findings, oxygen penetration depth and oxygen consumption rate can be great indicators of lean color and discoloration. However, when increases in oxygen concentrations are introduced into packaging systems, the increased partial pressure of oxygen increases and stabilizes the oxygen penetration depth and decreases the effectiveness of oxygen penetration depth as a tool to assess color stability (O'Keeffe and Hood, 1982). In addition, Madhavi and Carpenter (1993) stated that oxygen consumption rate alters the penetration of oxygen into the muscle. When there is a deep penetration of oxygen into the muscle there is a slower conversion of myoglobin to metmyoglobin, and when there is a shallow oxygen penetration into the muscle there is a rapid deterioration of the muscle.

Oxygen penetration depth is one of great variability. Trends were seen within all muscles as increases in oxygen penetration depth values were evaluated up until d 5 and then decreased to d 7 in the current study. Those muscles with high color stability (e.g., *m. longissimus lumborum* and *m. longissimus thoracis*) contained some of the highest oxygen penetration depths throughout the study. Moreover, those high color stability muscles also exhibited the least decrease in oxygen penetration depth. The large amount of variability between muscles presented in this study is supported by findings by Morley (1971). Hunt and Hendricks (1977) ranked various muscles on aerobic potential based on fiber characteristics. They reported the *m. psoas major* would have the greatest

followed by, in descending order outside *m. semitendinosus*, *m. longissimus dorsi*, *m. gluteus medius*, inside *m. semimembranosus*, and outside *m. semitendinosus*. This may help to explain the large oxygen penetration found in some of the *m. psoas major* samples and the *m. longissimus lumborum* and *m. longissimus thoracis* samples.

Hunter L Color Values (Lightness)*

The muscle × day interaction was significant (Figure 11). The *m. semitendinosus* muscle displayed the highest L* values during all retail display days, while the *m. supraspinatus* had the lowest L* values (Figure 11).

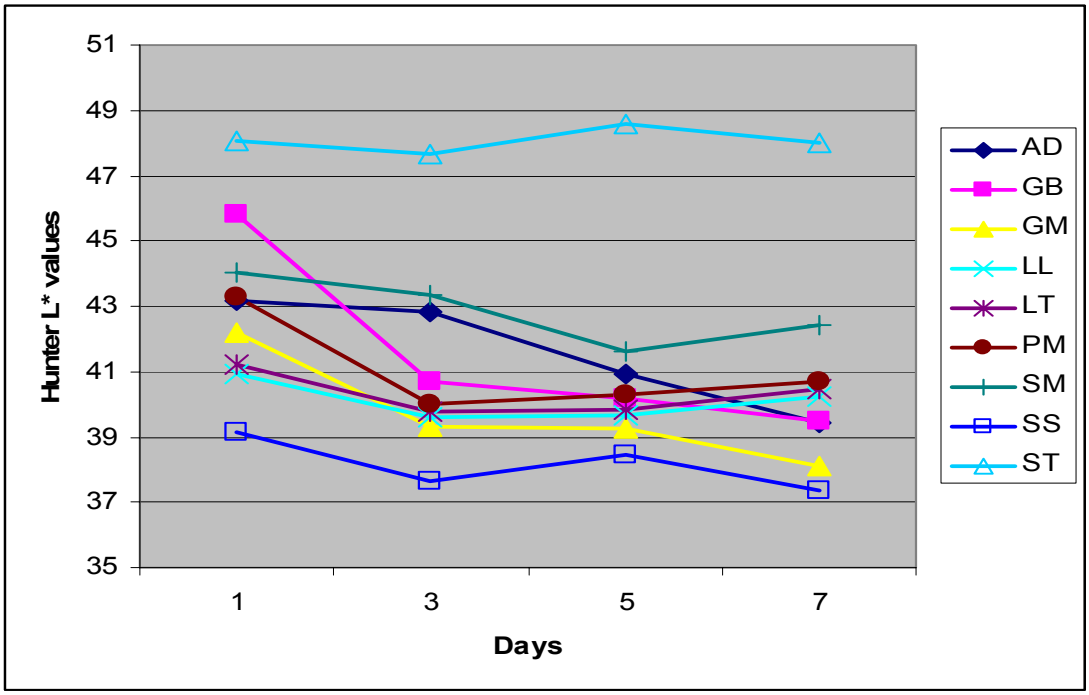

Figure 11. Least squares means for L* values for muscle × day interactions (P = 0.0011). AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*
*SEM is the standard error of least squares means (SEM = 0.707)

Muscles clearly reacted differently during retail display as the *m. adductor, m. gluteobiceps*, and *m. psoas major* presented high L* values on d 1, but declined rapidly by d 3. The *m. longissimus lumborum* and *m. longissimus thoracis* remained relatively constant and had no differences from d 1 to d 7.

Hunter a Values (Redness)*

Muscle × day interaction was significant as muscles reacted differently over display time. The *m. longissimus lumborum* and *m. longissimus thoracis* displayed the highest a* values on day 7, while all other muscles were significantly lower (Figure 12).

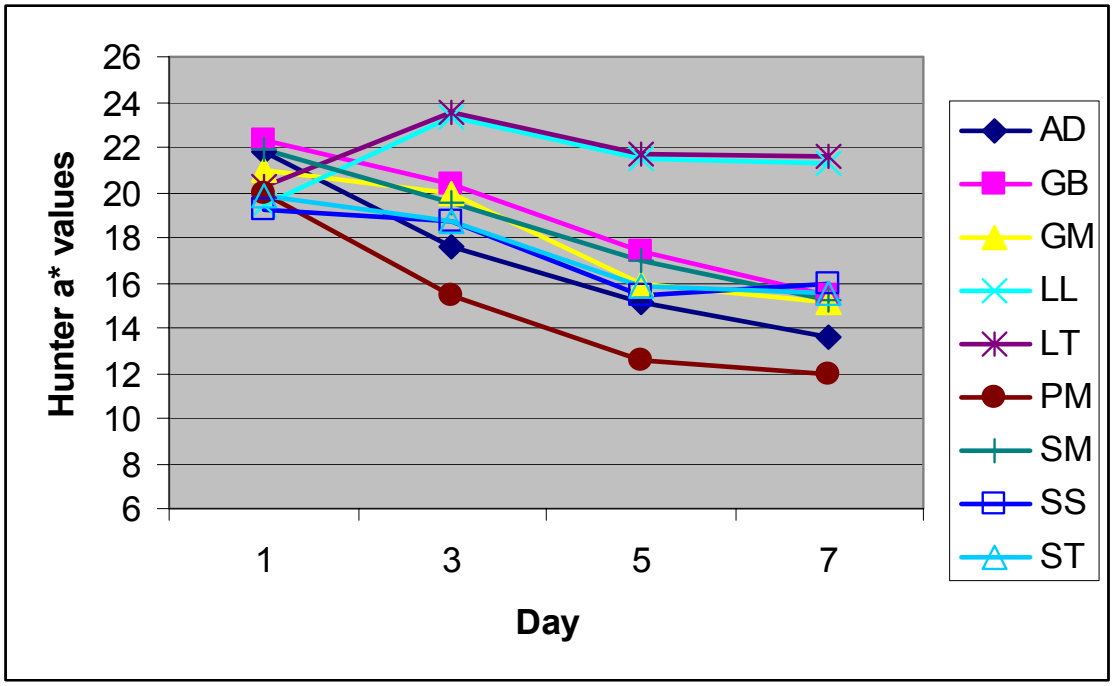

Figure 12. Least squares means for a* values for muscle × day interactions ($P < 0.0001$). AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*. *SEM is the standard error of least squares means (SEM = 0.631)

The m. adductor and *m. psoas major* exhibited the lowest a* values, which would be expected due to their poor color stability. In addition, the *m. adductor* and *m. psoas major* displayed a large decrease in a* values from d 1 to 3.

Hunter b Values (Yellowness)*

Overall the Select steaks had a higher b* value than Choice steaks (17.46 versus 16.72, respectively). The muscle × day interaction was significant, as muscles reacted differently during retail display (Figure 13).

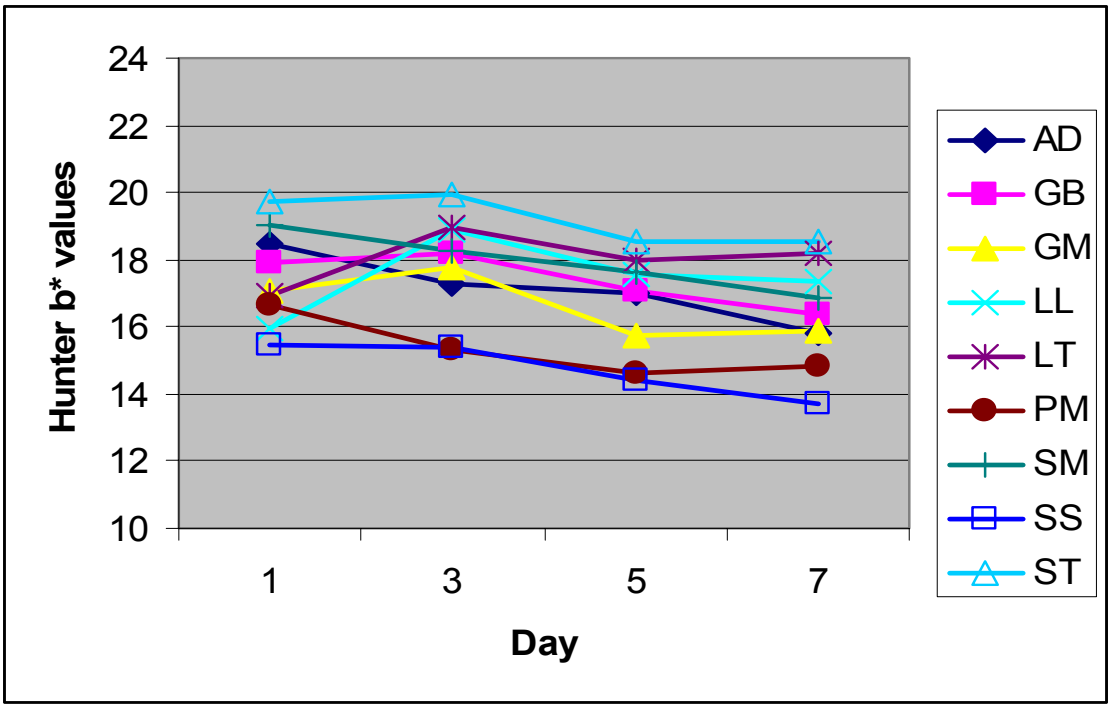

Figure 13. Least squares means for b* values for muscle × day interactions ($P < 0.0020$). AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*. *SEM is the standard error of least squares means (SEM = 0.397)

The *m. supraspinatus* and *m. psoas major* presented the lowest b* values, while the *m. semitendinosus*, *m. longissimus thoracis* and *m. longissimus lumborum* had the highest b* values on d 7. In addition, the *m. semitendinosus* had the least change over the 7 d retail display along with the *m. longissimus thoracis* (Figure 13).

The CIE Hunter L* and b* values were highly correlated with sensory lean color values, which have been shown to be effective in color sensory. Hunter L* values are commonly used to evaluate the lightness of steaks. This value is probably associated with the fiber characteristic. Also a* values (redness) also tends to be a predictor of color. In the current study a* values were highly correlated with sensory discoloration values. Bell et al. (1996) found that Hunter color values and visual analysis were unacceptable after 96 hr display; however, little differences in L* values were observed between products displayed for 2 and 98 hr. Generally, a* values decrease over storage and display, which also was found by Jeremiah and Gibson (2001). In addition, Bell and associates (1996) reported that a* values decreased during display and with the post-slaughter age of meat, which follows the same trend in the present study for *m. adductor*, *m. gluteobiceps*, *m. psoas major*, and *m. supraspinatus*. Jeremiah and Gibson (2001) reported that a* values were negatively related to the duration of storage as shown by a decrease in a* values as time increased. The current research displayed more constant values over display time for those muscles that were more color stable (e.g. *m. longissimus lumborum*), while muscle that were low color stability decreased in Hunter L*, a* and b* values.

Sensory Lean Color Evaluation

Muscle × day interaction was significant for sensory lean color values. The *m. semitendinosus* consistently had the highest lean color values (Figure 14), as they were brighter red. Moreover, the *m. longissimus lumborum* and *m. longissimus thoracis* displayed little change over the 7 d display period, while the *m. adductor*, *m. gluteobiceps*, *m. gluteus medius*, *m. psoas major* and *m. supraspinatus* showed the most decrease from d 1 to d 7 of display (Figure 14).

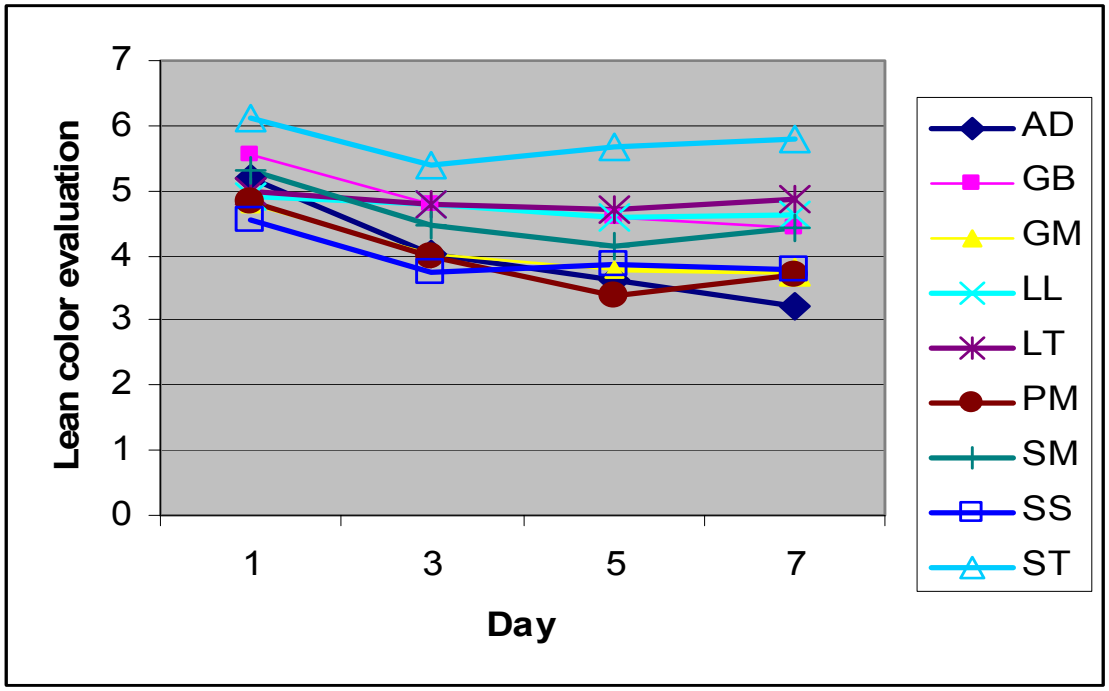

Figure 14. Least squares means for sensory lean color values for muscle × day interactions ($P < 0.0001$). (1 = extremely dark red; 8 = bright cherry-red) - AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*. *SEM is the standard error of least squares means (SEM = 0.111)

Sensory Discoloration Evaluation

There was a muscle × day interaction for discoloration as the *m. longissimus lumborum* and *m. longissimus thoracis* did not significantly change over the 7 d of display, while all other muscles increased in discoloration (Figure 15). The *m. psoas major* had the most discoloration by d 7 followed by the *m. adductor*, *m. gluteobiceps*, and *m. semimembranosus* (Figure 15). There was no difference between grades on d 1 and 3; however, Choice steaks became more discolored on d 5 and 7 than Select steaks from the same retail display (Figure 16).

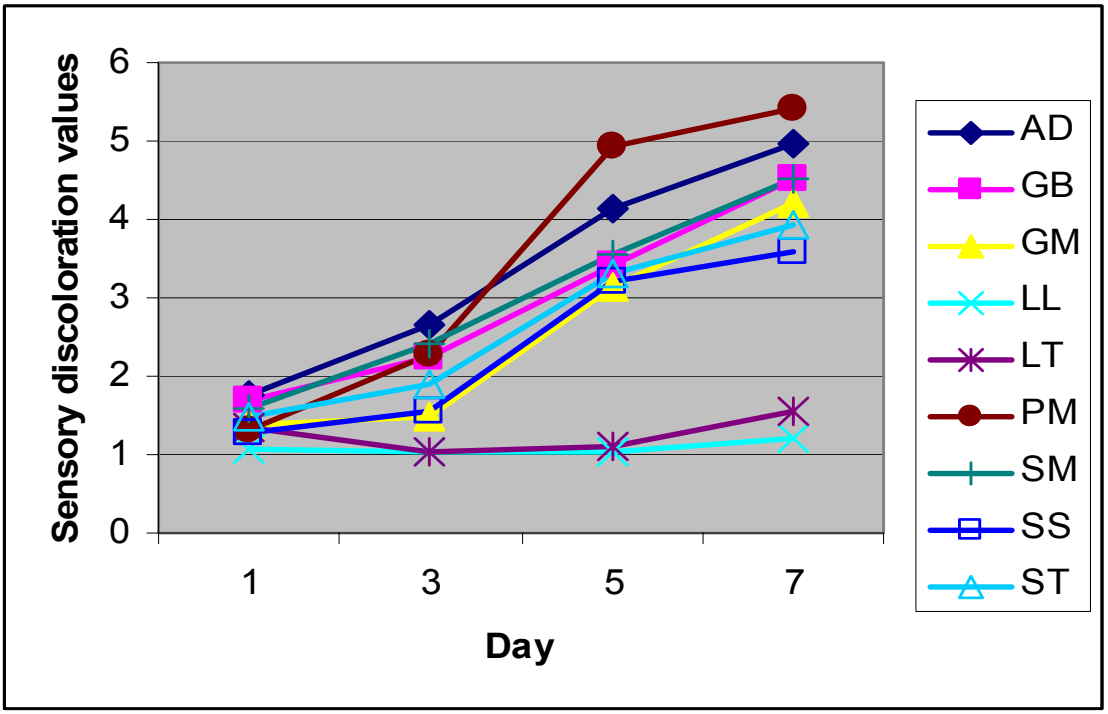

Figure 15. Least squares means for sensory discoloration values for muscle × day interactions ($P < 0.0001$). (1 = 0%discoloration; 7 = 100% discoloration) - AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*. *SEM is the standard error of least squares means (SEM = 0.141)

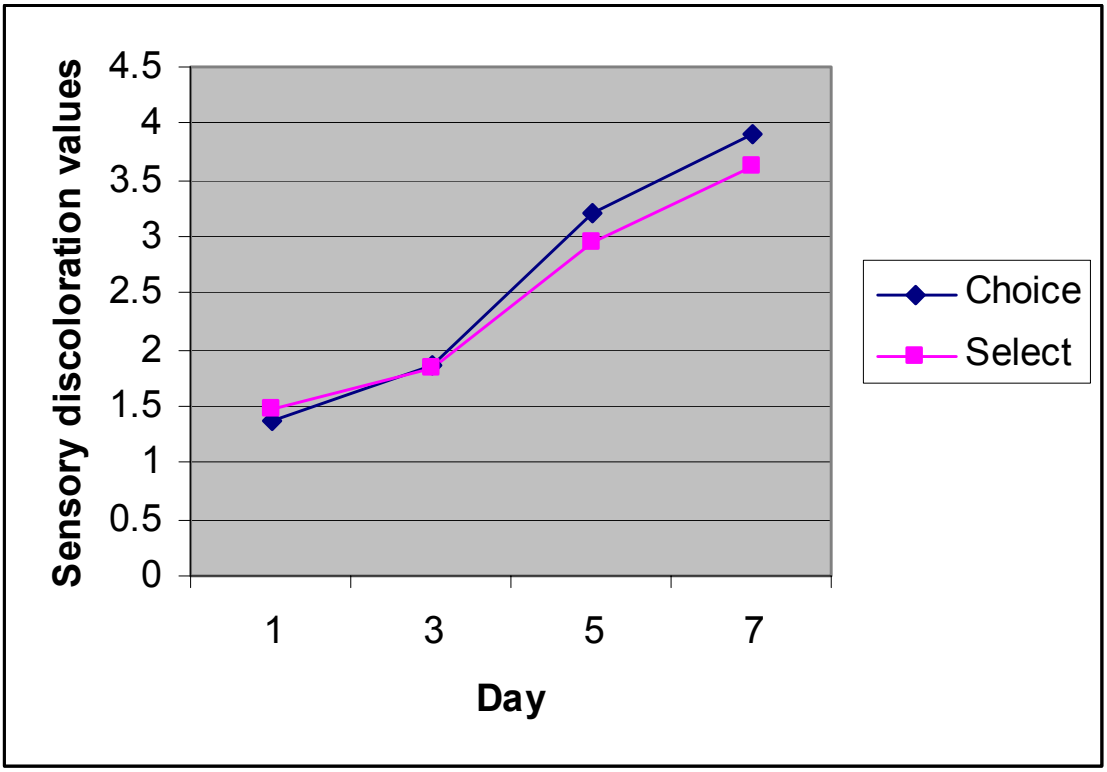

Figure 16. Least squares means for sensory discoloration values for day × grade interactions ($P = 0.0101$). (1 = 0% discoloration; 7 = 100% discoloration). *SEM is the standard error of least squares means (SEM = 0.067)

pH Values

Muscles were different in pH (Table 9). Those muscles with poor color stability (i. e., *m. supraspinatus* and *m. psoas major*) had the highest pH. Also, pH increased over display time (Table 10). The *m. adductor*, *m. gluteobiceps*, *m. gluteus medius*, *m. longissimus lumborum*, and *m. longissimus thoracis* had the largest increase in pH. There was no grade effect of Choice and Select steaks for pH (5.47 versus 5.48, respectively).

Table 9. Main effects of muscle on pH ($P < 0.0001$)

MUSCLE	pH
m. adductor	5.47[b]
m. gluteobiceps	5.42[e]
m. gluteus medius	5.44[cd]
m. longissimus lumborum	5.46[bc]
m. longissimus thoracis	5.47[b]
m. psoas major	5.57[a]
m. semimembranosus	5.43[de]
m. supraspinatus	5.59[a]
m. semitendinosus	5.43[de]
SEM*	0.007

[a-e]Means within the same column without a common superscript letter differ ($P < 0.05$)
*SEM is the standard error of least squares means

Table 10. Main effects of day on pH ($P < 0.0001$)

	Day				
	1	3	5	7	SEM*
pH	5.45[a]	5.47[ab]	5.48[b]	5.50[c]	0.005

[a-c]Means within the same column without a common superscript letter differ ($P < 0.05$)
*SEM is the standard error of least squares means

Many studies have elucidated that pH plays an important role in regulation of enzymes and muscle respiration post-mortem (Ledward, 1970; Bendall, 1972; and Bendall and Taylor, 1972). This has been reported to have a great effect on color stability. Ledward (1970) reported that reduction in pH caused a reduction in metmyoglobin reductase activity. The present study saw those muscles with high pH

(e.g., *m. psoas major* and *m. supraspinatus*) also had high metmyoglobin reductase activity; however, when this trend evaluated closer variability in metmyoglobin reductase activity did not follow and true trend in pH, as pH did not decline in most muscles. Moreover, as muscle becomes lower in ultimate pH values myoglobin is more susceptible to auto-oxidation and oxidation at accelerated levels (Ledward, 1972).

Correlation Coefficients

Correlation coefficients were evaluated to assess the relationship between measurements (Table 11). Hunter a* values correlated the most with discoloration (r = -0.73; $P < 0.0001$). As would be expected, a* values decreased as discoloration increased. Moreover, L* values were highly correlated with lean color evaluation (r = 0.56; $P < 0.0001$). Percent metmyoglobin correlated with discoloration, as would be expected (r = 0.32; $P < 0.0001$). Also, myoglobin content and TBARS values correlated with discoloration (r = -0.33, r = 0.42, respectively; $P < 0.0001$). Oxygen penetration depth was negatively correlated with discoloration, as oxygen penetration decreased, discoloration increased (r = -0.37; $P < 0.0001$). Metmyoglobin reductase activity was positively correlated with Hunter L* values (r = -0.29; $P < 0.0001$).

There continues to be a debate on whether reducing ability or oxygen consumption rate is the most determinant factor in color stability. Some researchers have reported that reducing ability plays a minimal effect on color stability (Atkinson and Follett, 1973; O'Keeffe and Hood, 1982), despite evidence that reducing ability is the most prominent factor in color stability (Ledward, 1972). Aerobic reducing ability was positively correlated with oxygen consumption rate in the present study, which

supports findings by Sammel et al. (2002). In addition, reducing ability in muscle was negatively correlated with discoloration in the present study, whereas reducing ability decreased as discoloration increased. Still, these factors are not highly correlated enough to accurately account for the entire amount of color stability found in different muscles. Cheah and Cheah (1971) reported that mitochondria maintain their oxidative capacity for six days postmortem. Realizing this it is possible to elucidate that if any of the muscles in the present study still contained any respiration by the mitochondria that it would be minimal as steaks were cut at 10 d postmortem.

Table 11. Correlation coefficients of biochemical, physical, and objective measurements

	MET	MYO	TBAR	MRA	ARA	OCR	OPD	L*	A*	B*	LEAN	DISC
MYO	-0.12 **											
TBAR	-0.03	-0.33 ***										
MRA	-0.11**	0.06	0.28 ***									
ARA	-0.10 **	0.07	-0.14 **	0.07								
OCR	-0.05	0.17 ***	-0.26 ***	-0.02	0.17 ***							
OPD	-0.21 ***	0.01	-0.18 ***	-0.16 ***	-0.11 **	-0.11 **						
L*	0.07	-0.09 *	-0.14 **	-0.29 ***	-0.14 **	0.08	0.03					
A*	-0.12 **	0.21 ***	-0.37 **	-0.12 **	0.05	0.17 ***	0.36 ***	0.02				
B*	0.00	-0.07	-0.14 **	-0.24 ***	-0.11 **	-0.01	0.31 ***	0.35 ***	0.66 ***			
LEAN	-0.18 ***	0.09 *	-0.23 ***	-0.13 **	-0.03	0.15 ***	0.15 ***	0.56 ***	0.05	0.42 ***		
DISC	0.32 ***	-0.33 ***	0.42 ***	0.03	-0.19 ***	-0.21 ***	-0.37 ***	-0.01	-0.73 ***	-0.29 ***	-0.42 ***	
PH	-0.06	-0.07	0.13 **	0.16 ***	0.06	-0.08	-0.04	-0.31 ***	-0.25 ***	-0.38 ***	-0.33 ***	0.13 **

MET = FAT = %Fat; MO = % Moisture; MET = Metmyoglobin; MYO = Myoglobin; TBARS = 2-thiobarbituric acid reactive substances (mg/kg); MRA = metmyoglobin reductase activity (nmoles/min·g); ARA = aerobic reducing ability (Δ% metmyoglobin); OCR = oxygen consumption rate; L* = Hunter lightness value; a* = Hunter redness value; b* = Hunter yellowness value, LEAN = sensory lean color values (1 = extremely dark red; 8 = bright cherry-red); DISC = sensory discoloration values (1 = 0%discoloration; 7 = 100% discoloration); pH.
P-value > 0.05 = "-";< 0.05 = "*"; < 0.01 = "**"; <0.0001 = "***"

CHAPTER V

SUMMARY, CONCLUSIONS, AND IMPLICATIONS

Color stability is dependent on many factors. This study agrees with much of the research which finds metmyoglobin reductase activity to be inefficient at predicting or determining color stability. Metmyoglobin reductase activity had very little to no relationship to meat color stability as found in the current research. There was a variation in color stability among the muscles evaluated in this research. Aerobic reducing ability looks to be a promising component of color stability because of the inherent metabolic differences between muscles.

The industry continues to extend color stability by as many days as possible, especially those very low color stability muscles (i.e., *m. psoas major*). As shown in this study, the oxidative potential of the muscle is an essential component to extending color stability. Thus, the strategy for maintaining optimum meat color involves the delay of pigment oxidation and the reduction of met-heme species.

Most research has concluded that the rate of pigment oxidation in meat was affected most by tissue oxygen consumption. Oxygen consumption rate was not measured accurately enough to assess differences between muscles, but did allow for speculation that after a few days of retail display, oxygen consumption has reduced to very low amounts. Oxygen penetration depth did show that those high color stability muscles (i.e., *m. longissimus lumborum*) remained high over display time, despite having

a high oxygen penetration, which was found initially in low color stability samples (i.e., *m. psoas major*).

Muscle clearly had a major impact on overall color stability; however, grade showed only few differences, which conflicted with results from previous research. The USDA Select steaks tended to be higher in color stability than USDA Choice. The data indicated that differences in muscle types and grades can play a major role in shelf-stability due to different oxidative potentials and reducing ability. Therefore, regardless of what variables are measured muscle type and grade remain complicated factors in the quest to better understand color stability.

As previously stated, muscle is a complicated system and conflicting research complicates the full understanding of what causes some muscles to convert myoglobin to metmyoglobin so rapidly, while others have a slower conversion rate. However, oxidative potential of individual muscles is clearly an important part of color stability. This study helps provide information towards solving color stability problems in some muscles. As the industry continues to bring more innovative cuts and muscles to the consumer, it will become more important to develop strategies for these muscles to prevent the onset of metmyoglobin formation. With better understanding of each muscle's biochemical and physical attributes, the industry can target individual muscles through processing or packaging strategies to help improve and maximize color shelf-life of individual muscles.

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APPENDIX I

SENSORY COLOR DATA COLLECTION

The following figures (I-1 and I-2) contain color data collection sheet and sensory color blocks used in the sensory color evaluation.

Date_____ **Recorder**_____

Color Evaluation

Sample #	Storage Day	Beef Color	Surface Discoloration	Comments

Beef Color

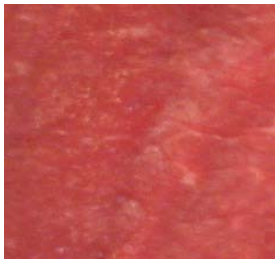
8 = Extremely bright cherry-red
7 = Bright cherry-red
6 = Moderately bright cherry-red
5 = Slightly bright cherry-red
4 = Slightly dark cherry-red
3 = Moderately dark red
2 = Dark red
1 = Extremely dark red

Surface Discoloration

7 = total discoloration (100%)
6 = Extensive discoloration (80 – 99%)
5 = Moderate discoloration (60 – 79%)
4 = Modest discoloration (40 – 59%)
3 = Small discoloration (20 – 39%)
2 = Slight discoloration (1 – 19%)
1 = No discoloration (0%)

Figure I-1. Sensory color evaluation data collection sheet.


8= Extremely bright cherry-red

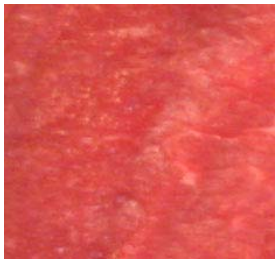

4= Slightly dark cherry-red


7= Bright cherry-red


3= Moderately dark red


6= Moderately bright cherry-red


2= Dark red


5= Slightly bright cherry-red

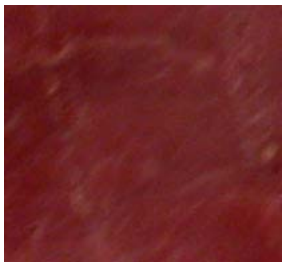

1= Extremely dark red

Figure I-2. Color blocks used for sensory color evaluation.

APPENDIX II

SIGNIFICANT INTERACTIONS

The following tables (II-1 through II-12) contain significant interactions in tabular form for percent fat, percent moisture, percent metmyoglobin, TBARS, myoglobin content, metmyoglobin reductase activity, aerobic reducing ability, oxygen consumption rate, oxygen penetration depth, Hunter L*, Hunter a*, Hunter b*, sensory lean color evaluation, sensory discoloration evaluation, and pH.

Table II-1. Least squares means for myoglobin content (mg/g) for muscle × day interactions ($P = 0.0032$)

DAY	AD[q]	GB[q]	GM[q]	LL[q]	LT[q]	PM[q]	SM[q]	SS[q]	ST[q]
1	4.70^{d-j}	5.98^{a-c}	6.17^{ab}	7.05^{a}	5.63^{b-d}	4.81^{d-i}	5.14^{b-h}	5.52^{b-f}	4.17^{g-m}
3	5.29^{b-g}	4.67^{d-j}	6.26^{ab}	7.18^{a}	5.55^{b-e}	3.95^{h-o}	5.12^{b-h}	4.47^{d-k}	3.45^{k-p}
5	4.33^{f-m}	4.00^{h-o}	4.13^{g-n}	4.94^{c-h}	4.12^{g-n}	3.50^{j-p}	4.07^{h-n}	4.42^{e-l}	3.20^{m-p}
7	3.69^{i-o}	2.93^{n-p}	3.66^{i-o}	3.25^{l-p}	2.82^{op}	2.32^{p}	3.40^{k-p}	3.56^{i-p}	3.44^{k-p}

[a-p]Means for myoglobin content without a common superscript letter differ ($P < 0.05$).

[q]AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*.

*SEM is the standard error of least squares means (SEM = 0.435)

Table II-2. Least squares means for TBARS (mg/kg) for day × grade interactions ($P < 0.0054$)

DAY	GRADE	
	Choice	Select
1	0.07[c]	0.07[c]
3	0.15[c]	0.14[c]
5	0.15[c]	0.12[c]
7	0.73[a]	0.54[b]

[a-d]Means for TBAR values without a common superscript letter differ ($P < 0.05$).
*SEM is the standard error of least squares means (SEM = 0.029)

Table II-3. Least squares means for aerobic reducing ability (Δ% metmyoglobin) for muscle × day interactions ($P < 0.0024$)

DAY	AD[o]	GB[o]	GM[o]	LL[o]	LT[o]	PM[o]	SM[o]	SS[o]	ST[o]
1	8.22^{ab}	6.45^{b-d}	6.50^{b-d}	2.65^{d-l}	0.98^{g-n}	8.17^{ab}	7.91^{a-c}	11.24^{a}	3.68^{d-j}
3	0.18^{h-n}	3.85^{c-i}	0.95^{g-n}	5.64^{b-e}	2.22^{e-m}	2.00^{e-m}	-2.83^{n}	3.27^{d-k}	1.17^{g-n}
5	3.39^{d-k}	5.51^{b-f}	1.71^{e-l}	4.48^{b-g}	4.25^{b-h}	1.33^{f-n}	0.74^{g-n}	3.48^{d-j}	1.09^{g-n}
7	0.62^{g-n}	-0.10^{i-n}	-0.29^{i-n}	-1.31^{l-n}	-0.77^{k-n}	1.77^{e-m}	-0.47^{j-n}	1.88^{e-l}	-1.58^{mn}

[a-n]Means for aerobic reducing ability without a common superscript letter differ ($P < 0.05$).

[o]AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*.

*SEM is the standard error of least squares means (SEM = 1.195)

Table II-4. Least squares means for oxygen penetration depth (mm) values for muscle × day interactions ($P < 0.0001$)

DAY	AD[s]	GB[s]	GM[s]	LL[s]	LT[s]	PM[s]	SM[s]	SS[s]	ST[s]
1	3.07^{qr}	3.06^{qr}	3.48^{n-r}	5.58^{d-h}	6.30^{d}	3.98^{l-p}	3.87^{m-q}	3.11^{p-r}	4.76^{h-l}
3	4.52^{j-m}	4.68^{i-m}	4.60^{i-m}	7.49^{c}	8.77^{ab}	6.21^{de}	5.01^{g-k}	4.58^{i-m}	5.91^{d-f}
5	4.92^{g-k}	4.23^{k-n}	5.10^{f-j}	8.10^{bc}	9.24^{a}	5.39^{e-i}	5.44^{e-i}	4.60^{i-m}	6.15^{de}
7	3.57^{n-r}	3.20^{o-r}	3.94^{l-p}	5.41^{e-i}	5.64^{d-g}	3.00^{r}	4.00^{l-o}	3.42^{n-r}	3.44^{n-r}

[a-r]Means for oxygen penetration depth values without a common superscript letter differ ($P < 0.05$).
[s]AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*.
SEM is the standard error of least squares means (SEM = 0.309)

Table II-5. Least squares means for oxygen penetration depth (mm) values for muscle × grade interactions ($P = 0.0424$)

GRADE	AD[g]	GB[g]	GM[g]	LL[g]	LT[g]	PM[g]	SM[g]	SS[g]	ST[g]
Choice	3.74[ef]	3.34[f]	4.05[d-f]	6.78[b]	6.71[b]	3.97[d-f]	4.42[c-e]	3.60[ef]	5.34[c]
Select	4.25[d-f]	4.24[d-f]	4.50[c-e]	6.50[b]	8.26[a]	5.32[c]	4.74[cd]	4.26[d-f]	4.79[cd]

[a-f]Means for oxygen penetration depth values without a common superscript letter differ ($P < 0.05$).

[g]AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*.

*SEM is the standard error of least squares means (SEM = 0.334)

Table II-6. Least squares means for oxygen penetration depth (mm) values for day × grade interactions ($P = 0.0498$)

DAY	1	3	5	7
Choice	4.07[c]	5.53[b]	5.52[b]	3.52[d]
Select	4.20[c]	5.97[a]	6.29[a]	4.38[c]

[a-d]Means for oxygen penetration depth values without a common superscript letter differ ($P < 0.05$).

*SEM is the standard error of least squares means (SEM = 0.146)

Table II-7. Least squares means for L* values for muscle × day interactions ($P = 0.0011$)

DAY	AD[o]	GB[o]	GM[o]	LL[o]	LT[o]	PM[o]	SM[o]	SS[o]	ST[o]
1	43.16^{d}	45.83^{bc}	42.22^{d-g}	40.93^{e-j}	41.20^{e-i}	43.28^{d}	44.02^{cd}	39.14^{j-n}	48.08^{a}
3	42.84^{de}	40.71^{f-j}	39.29^{i-n}	39.61^{i-m}	39.77^{h-l}	40.00^{h-l}	43.37^{d}	37.65^{mn}	47.68^{ab}
5	40.92^{e-j}	40.19^{h-k}	39.24^{j-n}	39.64^{i-l}	39.84^{h-l}	40.29^{g-k}	41.63^{d-h}	38.43^{k-n}	48.61^{a}
7	39.42^{i-n}	39.47^{i-m}	38.08^{l-n}	40.21^{h-k}	40.48^{f-j}	40.67^{f-j}	42.44^{d-f}	37.38^{n}	47.99^{a}

[a-n]Means for L* values without a common superscript letter differ ($P < 0.05$).

[o]AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*.

*SEM is the standard error of least squares means (SEM = 0.707)

Table II-8. Least squares means for a* values for muscle × day interactions ($P < 0.0001$)

DAY	AD[p]	GB[p]	GM[p]	LL[p]	LT[p]	PM[p]	SM[p]	SS[p]	ST[p]
1	21.80[a-d]	22.30[a-c]	21.00[c-g]	19.39[gh]	20.24[d-h]	19.96[e-h]	21.86[a-d]	19.26[g-i]	19.86[f-h]
3	17.54[i-k]	20.33[d-h]	19.90[f-h]	23.34[ab]	23.51[a]	15.45[lm]	19.52[gh]	18.71[h-j]	18.71[h-j]
5	15.12[mn]	17.37[jk]	15.98[k-m]	21.44[c-f]	21.69[b-e]	12.59[o]	16.97[j-l]	15.40[lm]	15.88[k-m]
7	13.60[no]	15.48[lm]	15.15[mn]	21.32[c-f]	21.54[c-f]	11.98[o]	15.21[mn]	15.91[k-m]	15.49[lm]

[a-o]Means for a* values without a common superscript letter differ ($P < 0.05$).

[p]AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*.

*SEM is the standard error of least squares means (SEM = 0.631)

Table II-9. Least squares means for b* values for muscle × day interactions ($P < 0.0020$)

DAY	AD[r]	GB[r]	GM[r]	LL[r]	LT[r]	PM[r]	SM[r]	SS[r]	ST[r]
1	$18.45^{b\text{-}f}$	$17.89^{c\text{-}i}$	$17.07^{f\text{-}l}$	$15.96^{k\text{-}o}$	$16.94^{g\text{-}l}$	$16.62^{i\text{-}n}$	$19.01^{a\text{-}c}$	$15.47^{m\text{-}p}$	19.72^{ab}
3	$17.27^{e\text{-}k}$	$18.19^{c\text{-}h}$	$17.78^{c\text{-}i}$	$18.89^{a\text{-}d}$	$18.94^{a\text{-}d}$	$15.33^{n\text{-}p}$	$18.29^{c\text{-}g}$	$15.39^{n\text{-}p}$	19.96^{a}
5	$16.99^{g\text{-}l}$	$17.07^{f\text{-}l}$	$15.76^{l\text{-}p}$	$17.58^{d\text{-}j}$	$18.01^{c\text{-}i}$	$14.60^{o\text{-}q}$	$17.65^{c\text{-}j}$	14.38^{pq}	$18.56^{b\text{-}e}$
7	$15.79^{l\text{-}o}$	$16.34^{j\text{-}n}$	$15.87^{l\text{-}o}$	$17.37^{e\text{-}j}$	$18.17^{c\text{-}h}$	$14.80^{o\text{-}q}$	$16.84^{h\text{-}m}$	13.70^{q}	$18.55^{b\text{-}e}$

[a-q]Means for b* values without a common superscript letter differ ($P < 0.05$).

[r]AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*.

*SEM is the standard error of least squares means (SEM = 0.397)

Table II-10. Least squares means for sensory lean color values for muscle × day interactions ($P < 0.0001$) (1 = extremely dark red; 8 = bright cherry-red)

DAY	AD[r]	GB[r]	GM[r]	LL[r]	LT[r]	PM[r]	SM[r]	SS[r]	ST[r]
1	5.18[ef]	5.54[b-d]	4.78[g-i]	4.91[f-h]	4.98[fg]	4.84[g-i]	5.32[de]	4.54[jk]	6.13[a]
3	4.04[lm]	4.80[g-i]	4.02[l-n]	4.77[g-i]	4.78[g-i]	3.99[l-n]	4.48[jk]	3.76[m-o]	5.41[c-e]
5	3.63[op]	4.59[ij]	3.77[m-o]	4.57[ij]	4.71[g-j]	3.39[pq]	4.16[kl]	3.86[l-o]	5.66[bc]
7	3.20[q]	4.41[jk]	3.74[m-o]	4.61[h-j]	4.85[g-i]	3.72[no]	4.42[jk]	3.78[m-o]	5.79[b]

[a-q]Means for lean color evaluation without a common superscript letter differ ($P < 0.05$).
[r]AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*.
*SEM is the standard error of least squares means (SEM = 0.111)

Table II-11. Least squares means for sensory discoloration values for muscle × day interactions ($P < 0.0001$) (1 = 0%discoloration; 7 = 100% discoloration)

DAY	AD[p]	GB[p]	GM[p]	LL[p]	LT[p]	PM[p]	SM[p]	SS[p]	ST[p]
1	1.76^{kl}	1.68^{k-m}	1.37^{l-o}	1.07^{o}	1.33^{m-o}	1.31^{m-o}	1.58^{k-n}	1.29^{no}	1.50^{l-n}
3	2.64^{h}	2.24^{ij}	1.49^{l-n}	1.05^{o}	1.03^{o}	2.29^{h-j}	2.43^{hi}	1.54^{k-n}	1.91^{jk}
5	4.14^{cd}	3.40^{fg}	3.15^{g}	1.03^{o}	1.09^{o}	4.94^{b}	3.54^{fg}	3.21^{fg}	3.30^{fg}
7	4.96^{b}	4.53^{c}	4.21^{cd}	1.21^{no}	1.56^{k-n}	5.42^{a}	4.53^{c}	3.58^{ef}	3.94^{de}

[a-o]Means for sensory discoloration values without a common superscript letter differ ($P < 0.05$).

[p]AD = *m. adductor*; GB = *m. gluteobiceps*; GM = *m. gluteus medius*; LL = *m. longissimus lumborum*; LT = *m. longissimus thoracis*; PM = *m. psoas major*; SM = *m. semimembranosus*; SS = *m. supraspinatus*; ST = *m. semitendinosus*.

*SEM is the standard error of least squares means (SEM = 0.141)

Table II-12. Least squares means for sensory discoloration values for day × grade interactions ($P = 0.0101$) (1 = 0% discoloration; 7 = 100% discoloration)

	1	3	5	7
Choice	1.38[f]	1.85[e]	3.21[c]	3.91[a]
Select	1.48[f]	1.84[e]	2.96[d]	3.62[b]

[a-f]Means for sensory discoloration values without a common superscript letter differ ($P < 0.05$).

*SEM is the standard error of least squares means (SEM = 0.067)

APPENDIX III

SIGNIFICANT INTERACTIONS

The following are ANOVA tables (III-1 through III-14) for percent fat, percent moisture, percent metmyoglobin, TBARS, myoglobin content, metmyoglobin reductase activity, aerobic reducing ability, oxygen consumption rate, oxygen penetration depth, Hunter L*, Hunter a*, Hunter b*, sensory lean color evaluation, sensory discoloration evaluation, and pH.

ANOVA III-1. Table for percent fat

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	17	159.95	9.41	9.62	<0.0001
Muscle	8	117.00	14.62	14.95	<0.0001
Grade	1	26.28	26.28	26.86	<0.0001
Muscle × Grade	8	16.67	2.08	2.13	0.0365
Error	144	140.84	0.98		
Corrected total	161	300.79			

ANOVA III-2. Table for percent moisture

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	17	162.95	9.59	7.08	<0.0001
Muscle	8	119.20	14.90	11.01	<0.0001
Grade	1	25.67	25.67	18.97	<0.0001
Muscle × Grade	8	18.27	2.28	1.69	0.1062
Error	144	194.87	1.35		
Corrected total	161	357.82			

ANOVA III-3. Table for percent metmyoglobin

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	164	168160.82	1025.37	3.28	<0.0001
Whole-plot					
Grade	1	8.92	8.92	0.01	0.9202
Carcass (Grade)	16	13765.72	860.36	2.75	0.0003
1st Split					
Muscle	8	14692.11	1836.51	6.61	<0.0001
Muscle × Grade	8	1672.91	209.11	0.75	0.6449
Muscle × Carcass(Grade)	128	35561.37	277.82	0.89	0.7874
2nd Split					
Day	3	100859.83	33619.94	107.61	<0.0001
Error	483	150900.94	312.42		
Corrected total	647	319061.76			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

ANOVA III-4. Table for myoglobin content

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	188	1359.88	7.23	2.13	<0.0001
Whole-plot					
Grade	1	29.96	29.96	5.54	0.0317
Carcass (Grade)	16	86.50	5.41	1.59	0.0677
1st Split					
Muscle	8	225.11	28.14	10.36	<0.0001
Muscle × Grade	8	31.23	3.90	1.44	0.1872
Muscle × Carcass(Grade)	128	347.74	2.72	0.80	0.9369
2nd Split					
Day	3	469.20	165.40	48.62	<0.0001
Muscle × Day	24	140.34	5.85	1.72	0.0192
Error	459	1561.61	3.40		
Corrected total	647	2921.49			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

ANOVA III-5. Table for TBARS

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	167	49.63	0.30	4.24	<0.0001
Whole-plot					
Grade	1	0.50	0.50	0.75	0.4007
Carcass (Grade)	16	10.64	0.66	9.48	<0.0001
1st Split					
Muscle	8	0.57	0.71	2.52	0.0142
Muscle × Grade	8	0.40	0.05	1.78	0.0871
Muscle × Carcass(Grade)	128	3.63	0.03	0.40	1.0000
2nd Split					
Day	3	32.94	10.98	156.58	<0.0001
Day × Grade	3	0.90	0.30	4.28	0.0054
Error	480	33.66	0.07		
Corrected total	647	83.29			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

ANOVA III-6. Table for metmyoglobin reductase activity

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	188	2414177.53	12841.37	4.34	<0.0001
Whole-plot					
Grade	1	32297.90	32297.90	0.35	0.5630
Carcass (Grade)	16	1480863	92553.99	31.28	<0.0001
1st Split					
Muscle	8	215766.36	26970.79	11.89	<0.0001
Muscle × Grade	8	14038.12	1754.77	0.77	0.6264
Muscle × Carcass(Grade)	128	290260.74	2267.66	0.77	0.9641
2nd Split					
Day	3	253503.76	84501.25	28.56	<0.0001
Muscle × Day	24	73745.56	3072.73	1.04	0.4141
Error	446	1319473.49			
Corrected total	634	3733651.02			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

ANOVA III-7. Table for aerobic reducing ability

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	188	16972.49	90.28	2.19	<0.0001
Whole-plot					
Grade	1	247.44	247.44	0.56	0.4649
Carcass (Grade)	16	7063.67	441.48	10.69	<0.0001
1st Split					
Muscle	8	928.83	116.10	4.65	<0.0001
Muscle × Grade	8	232.90	29.11	1.17	0.3250
Muscle × Carcass(Grade)	128	3197.07	24.98	0.60	0.9996
2nd Split					
Day	3	3302.29	1100.77	26.64	<0.0001
Muscle × Day	24	2046.65	85.28	2.06	0.0024
Error	459	18963.91	41.32		
Corrected total	647	35936.41			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

ANOVA III-8. Table for oxygen consumption rate

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	164	193.77	1.18	1.77	<0.0001
Whole-plot					
Grade	1	1.27	1.27	1.64	0.2188
Carcass (Grade)	16	12.42	0.78	1.16	0.2941
1st Split					
Muscle	8	3.19	0.40	0.67	0.7190
Muscle × Grade	8	2.87	0.36	0.60	0.7760
Muscle × Carcass(Grade)	128	76.46	0.60	0.89	0.7742
2nd Split					
Day	3	97.15	32.38	48.52	<0.0001
Error	483	322.40	0.67		
Corrected total	647	516.17			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

ANOVA III-9. Table for oxygen penetration depth

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	191	2481.95	12.99	7.56	<0.0001
Whole-plot					
Grade	1	48.39	48.39	3.03	0.1009
Carcass (Grade)	16	255.42	15.96	9.29	<0.0001
1st Split					
Muscle	8	960.75	120.09	29.88	<0.0001
Muscle × Grade	8	66.86	8.36	2.08	0.0424
Muscle × Carcass(Grade)	128	514.41	4.02	2.34	0.0001
2nd Split					
Day	3	522.69	174.23	101.35	<0.0001
Day × Grade	3	13.55	4.52	2.63	0.0498
Muscle × Day	24	106.62	4.44	2.58	<0.0001
Error	456	783.88	1.72		
Corrected total	647	3265.83			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

ANOVA III-10. Table for Hunter L* values

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	188	10940.19	58.19	6.46	<0.0001
Whole-plot					
Grade	1	429.44	429.44	2.49	0.1341
Carcass (Grade)	16	2759.44	172.46	19.16	<0.0001
1st Split					
Muscle	8	4548.66	568.58	37.29	<0.0001
Muscle × Grade	8	144.06	18.01	1.18	0.3154
Muscle × Carcass(Grade)	128	1951.47	15.25	1.69	0.0001
2nd Split					
Day	3	571.91	190.64	21.17	<0.0001
Muscle × Day	24	476.76	19.86	2.21	0.0010
Error	458	4123.66	9.00		
Corrected total	646	15063.85			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

ANOVA III-11. Table for Hunter a* values

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	188	7852.73	41.77	5.82	<0.0001
Whole-plot					
Grade	1	41.90	41.90	0.69	0.4196
Carcass (Grade)	16	976.75	61.05	8.51	<0.0001
1st Split					
Muscle	8	2572.74	321.59	58.38	<0.0001
Muscle × Grade	8	44.21	5.53	1.00	0.4369
Muscle × Carcass(Grade)	128	705.10	5.51	0.77	0.9634
2nd Split					
Day	3	2189.17	729.72	101.73	<0.0001
Muscle × Day	24	1289.11	53.71	7.49	<0.0001
Error	458	3285.15	7.17		
Corrected total	646	11137.88			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

ANOVA III-12. Table for Hunter b* values

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	188	2315.34	12.32	2.75	<0.0001
Whole-plot					
Grade	1	89.91	89.91	5.34	0.0345
Carcass (Grade)	16	269.33	16.83	3.76	<0.0001
1st Split					
Muscle	8	1079.08	134.88	40.84	<0.0001
Muscle × Grade	8	11.82	1.48	0.45	0.8904
Muscle × Carcass(Grade)	128	422.77	3.30	0.74	0.9799
2nd Split					
Day	3	201.62	67.21	15.03	<0.0001
Muscle × Day	24	224.99	9.37	2.10	0.0020
Error	458	2047.76	4.47		
Corrected total	646	4363.09			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

ANOVA III-13. Table for sensory lean color evaluation

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	188	423.33	2.25	10.12	<0.0001
Whole-plot					
Grade	1	13.78	13.78	4.33	0.0539
Carcass (Grade)	16	50.89	3.18	14.30	<0.0001
1st Split					
Muscle	8	200.50	25.06	75.93	<0.0001
Muscle × Grade	8	1.35	0.17	0.51	0.8460
Muscle × Carcass(Grade)	128	42.25	0.33	1.48	0.0018
2nd Split					
Day	3	80.13	25.71	120.03	<0.0001
Muscle × Day	24	32.79	1.32	5.95	<0.0001
Error	459	102.14	0.22		
Corrected total	647	525.46			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

ANOVA III-14. Table for sensory discoloration evaluation

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	191	1312.85	6.87	19.00	<0.0001
Whole-plot					
Grade	1	2.07	2.07	0.44	0.5164
Carcass (Grade)	16	75.36	4.71	13.02	<0.0001
1st Split					
Muscle	8	416.48	52.06	114.44	<0.0001
Muscle × Grade	8	3.41	0.43	0.94	0.4878
Muscle × Carcass(Grade)	128	58.23	0.45	1.26	0.0467
2nd Split					
Day	3	569.19	189.73	524.36	<0.0001
Day × Grade	3	4.14	1.38	3.82	0.0101
Muscle × Day	24	182.37	7.60	21.00	<0.0001
Error	456	165.00	0.36		
Corrected total	647	1477.85			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

ANOVA III-15. Table for pH

Source	DF	Sum of Squares	Mean Squares	F Value	Pr > F
Model	164	3.35	0.02	4.27	<0.0001
Whole-plot					
Grade	1	0.004	0.004	0.17	0.6850
Carcass (Grade)	16	0.39	0.02	5.14	<0.0001
1st Split					
Muscle	8	2.19	0.27	69.90	<0.0001
Muscle × Grade	8	0.03	0.004	1.07	0.3905
Muscle × Carcass(Grade)	128	0.50	0.004	0.82	0.9105
2nd Split					
Day	3	0.19	0.06	13.42	<0.0001
Error	483	2.31	0.005		
Corrected total	647	5.65			

Whole-plot used Type III mean squares of Carcass(Grade) as an error term
1st Split used Type III mean squares of Muscle × Carcass(Grade) as an error term

VITA

Jason Monroe Behrends, son of Monroe A. and Karen A. (Dietert) Behrends, was born November 1, 1976, in Fredericksburg, Texas. He graduated from Fredericksburg High School, Fredericksburg, TX in May 1995. After high school he entered Texas Tech University, where he was a member of the 1997 National Champion Meat Judging Team, as well as the 1996 Wool Judging Team and 1998 Spring Livestock Judging Team. He coached the 1999 Texas Tech University Wool Judging Team. He graduated with a Bachelor of Science degree in animal science in May of 1999. He then entered graduate school at the University of Kentucky in June of 1999 pursuing a Master of Science in animal science with an emphasis in meat science. While at the University of Kentucky he coached the 2000 Meat Judging Team and was President of the Animal Sciences Graduate Student Association. He graduated with a Master of Science degree in animal science with an emphasis in meat science in May of 2001. He then entered graduate school at Texas A&M University in August of 2001 pursuing a Ph.D. in animal science with an emphasis in meat science and muscle biology. While at Texas A&M University he was Southern Regional Director and Treasurer of the first American Meat Science Association Student Board.

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